

THE CHEMOTHERAPY OF
PORCINE HEREDITARY LYMPHOSARCOMA

By

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Preface

The work described in this thesis was financed by the Cancer Research Campaign and carried out in the Department of Veterinary Medicine, Royal (Dick) School of Veterinary Studies, University of Edinburgh.

Part of the results have been published and reprints of the papers are included in Appendix V.

A summary of the work was presented as a paper at a joint meeting of the British Association of Cancer Research and the Royal Society of Medicine, London, October 1977 and was published in the Proceedings in the British Journal of Cancer.

Further studies on combination chemotherapy and the effects of razoxane (ICRF - 159) were also carried out but are not included to save excessive length.

This thesis has been composed by the author and is a record of original work carried out by the author in association with other members of a research group. It has not been submitted in any form for any other degree or professional qualification.

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SUMMARY

A hereditary form of lymphosarcoma in Large White pigs, associated with an autosomal recessive gene, is diagnosed usually before 3 months of age and is fatal by 15 months. The suitability of this condition for testing anticancer chemotherapeutic agents has been investigated using four agents representing each of the four major modes of action. Prednisolone, adriamycin (doxorubicin), cyclophosphamide and cytarabine as single agents were administered to normal pigs to determine appropriate dose levels and then to lymphosarcoma cases, and the results compared with previously reported effects in man and other species.

Prednisolone produced a reduction in numbers of circulating lymphocytes and in size of lymph nodes, which was striking in the lymphosarcomatous pigs, an increase in serum albumin and decrease in serum globulins and involution of the thymus with "overshoot" on recovery. The lymphosarcoma cases showed improvements in appetite and vigour and in neutrophil, thrombocyte and red cell counts.

Adriamycin was cumulatively toxic at high dose levels, producing bone marrow depression, involving all types of white blood cells, red cells and thrombocytes, gastro-intestinal damage, stunting of growth, alopecia, stomatitis, sclerosis of blood vessels and possibly cardiotoxicity. Lymphosarcoma cases did not

survive a dose level of 2mg/kg. One of these died of liver failure. A dose-level of 1mg/kg produced a general improvement in fitness, body condition and weight gain with temporary suppression of the tumour after each dose.

Cyclophosphamide produced few effects, other than a consistent reversible, neutropenia during the 1st week after dosing, even at high dose-levels. Alopecia and haemorrhagic cystitis were not seen. Depression of skin delayed hypersensitivity response and thymus involution were unexpected results. Lymphosarcoma cases showed evidence of temporary suppression of the tumour after each dose.

Normal pigs given cytarabine showed slight weight loss and progressive bone marrow depression. Anti-tumour effect was disappointing, only one lymphosarcomatous animal showing evidence of response, despite the fact that levels of deoxycytidine kinase, which activates cytarabine, were shown to be four times higher in lymphoid tissue from the lymphosarcoma cases compared with the normal pigs.

Further studies were an investigation of the chemotactic response to casein of blood monocytes and neutrophils from normal pigs and lymphosarcoma cases, both untreated and treated with prednisolone.

INTRODUCTION

Tumours of the Haematopoietic System

Lymphocytes are formed chiefly in the lymph nodes, spleen, Peyer's patches, and to a lesser extent in other sites, such as the bone marrow. The other types of leukocyte, the neutrophils, eosinophils, basophils, (granulocytes) and monocytes, and the red blood cells, are formed in the bone marrow. Precursors of any of these cells may be affected by neoplastic transformation, and the result is a tumour, the abnormal cells of which resemble the cell which originally became neoplastic. This may occur at any stage of differentiation from the primitive stem cell to the mature cell.

When abnormal white cells appear in the peripheral blood, the condition is called "leukaemia". This term is used to classify the range of diseases which originate from the haematopoietic cell precursors of the bone marrow or lymph nodes, and in which tumour cells circulate in the blood early in the disease. If the disease develops rapidly, i.e. if it is acute, the tumour cells tend to be immature and are often called "blast" cells e.g. acute lymphoblastic leukaemia. If the disease develops slowly i.e. if it is chronic, the cells tend to be more mature.

The type of disease is diagnosed by examination of peripheral blood smears and bone marrow samples, stained to demonstrate features of the different kinds of cell.

Solid tumours of the lymphoid system also occur. These are called "lymphomas". They are initially localised, usually in haematopoietic, but sometimes in non-haematopoietic, areas of the body. From the initial site, they spread by the lymphatics and by the bloodstream, to involve other lymph nodes, bone marrow and organs such as the liver, gastro-intestinal tract, and brain. At this stage, abnormal lymphocytes may appear in the peripheral blood, and the disease will resemble, and in some cases will be almost indistinguishable from leukaemia. Lymphomas are usually diagnosed by histopathologic examination of lymph node or other tissue.

The terms "lymphocytic leukaemia" and "lymphoma" in the past have been used indiscriminately. However, studies of these two types of disease in man have indicated that they may be very different. Lingeman (1969) discussed the differences between the leukaemias and the lymphomas and the epidemiological patterns of these diseases in different parts of the world. She stated that, although a wide range of cell types of leukaemias and lymphomas is seen, the most frequent types fit into fairly well-defined clinical and pathological syndromes, and the epidemiology of each of these has been investigated. For example, in the United States, leukaemias occur in definite age-associated distributions. Acute lymphocytic or lymphoblastic leukaemias are seen mainly in children and young adults, whereas acute myelocytic leukaemias most commonly occur in adults. Chronic

granulocytic leukaemia is commoner in young and middle-aged adults, whereas chronic lymphocytic leukaemia is rarely seen in people under the age of 40 years.

Because of the desire to treat these diseases in man, much attention has been devoted to classification, so that the epidemiology and natural progression of each type could be identified, and results of treatment assessed. However, there is still no general agreement on the classification of either the leukaemias or the lymphomas. Recent proposals for classification of the acute leukaemias were made by Bennett, Catovsky, Daniel, Flandrin, Galton, Gralnick and Sultan (1976).

Lymphomas in man

1. Hodgkin's disease Named after Thos. Hodgkin, an English physician (1798 - 1866).

The classical feature of this disease is the presence of Reed-Sternberg giant cells in histological sections of lymph nodes. The distinctive histology of the disease was first recognised by Greenfield (1878) but Sternberg (1898) and Dorothy Reed (1902) have been credited with

the discovery and the typical giant cells have been named after them. The tumour is usually restricted initially to one group of lymph nodes and spreads contiguously from one node to another, becoming widespread in the body late in the disease. The age groups in which the disease is more common are 20 to 30 years and 60 to 70 years. The tumour is very radiosensitive and is usually treated with x-rays, unless fairly advanced, but most cases are also given drugs.

2. Non-Hodgkin's lymphoma.

Following the recognition of Hodgkin's disease, the non-Hodgkin lymphomas were identified, and a great deal of literature on the subject has been published over the years. In many reports the term "lymphosarcoma" has been used to describe cases which were probably of this type. Non-Hodgkin's lymphomas usually involve bone marrow and other organs, mainly spleen, liver and groups of lymph nodes, very early in the course of the disease. The incidence increases with age.

There are two important methods commonly used to describe the varieties of the disease - the histopathological type and the extent of the disease at diagnosis.

1. Histopathological features of the disease:

Most authors now classify the non-Hodgkins lymphomas in accordance with Rappaport, Winter and Hicks (1956). Confusion over terminology makes many pre-1956 reports of doubtful relevance, but two large surveys which cannot be ignored are those of Gall and Mallory (1942) who compiled results from 618 cases of malignant lymphoma, 389 of

which had non-Hodgkin's type and Rosenberg, Diamond and Craven (1960), who reviewed 1269 patients with lymphosarcoma. Rappaport, et al., (1956) used the following criteria in their classification:-

- a) Cell type - lymphocytic or histiocytic.
- b) State of cell differentiation.
 - i. Well differentiated i.e. small cells with the typical appearance of lymphocytes.
 - ii. Poorly differentiated i.e. large cells which look immature (sometimes called lymphoblastic).
 - iii. Undifferentiated.
- c) Presence or absence of distinguishable normal lymph node architecture i.e. presence or absence of lymph nodules or follicles. If present, the disease is termed "nodular". If absent the term "diffuse" is used.

This classification has greatly helped to standardise nomenclature, but it is not universally accepted (Lukes and Collins, 1975).

Recent immunological techniques have made it possible to define the origin of the tumour cell in many cases, and a new classification based on the function of the cells is now being widely used. Nevertheless, the prognostic value of the Rappaport classification, has been confirmed in several other large series. Jones, Fuks, Bull, Kadin, Dorfman, Kaplan, Rosenberg and Kim (1973) studied 405

cases, Patchefsky, Brodsky, Menduke, Southard, Brooks, Nicklas and Hoch, (1974) 293 cases, Brown, Peters, Bergsagel and Reid (1975) 460 cases, and Dumont, Duffillot, Flandrin, Chelloul, Tristant and Bernard (1975) 244 cases.

II. Staging: Another major factor determining the prognosis in the non-Hodgkin's lymphomas is the extent of the disease when diagnosed. Staging is usually determined according to the Ann Arbor Classification, which was proposed for Hodgkin's disease (Carbone, Kaplan, Musshoff, Smithers and Tubiana 1971). With this classification, patients are given a) a clinical stage, based on history, physical examination, initial biopsy evidence of lymphoma and radiography, and b) a pathologic stage which is derived from histological findings after special surgical procedures such as exploratory laparotomy, and biopsies of various organs and bone marrow.

Table I shows the Ann Arbor Staging Classification for non-Hodgkin's lymphomas (as modified by Jones et al., 1973). Accurate determination of the stage of disease is important when deciding on treatment. In the series of Jones et al., (1973) the final pathologic stage based on the biopsy specimens (e.g. bone marrow) was more advanced than was indicated by the clinical assessment in 46% of their patients.

TABLE I.Ann Arbor Staging Classification - non-Hodgkin's lymphoma.Clinical Staging (CS)

Stage I: Involvement of a single lymph node region (I) or of a single extralymphatic organ or site (I_E).

Stage II: Involvement of two or more lymph node regions on the same side of the diaphragm (II), or localised involvement of an extralymphatic organ or site and of one or more lymph node regions on the same side of the diaphragm (II_E).

Stage III: Involvement of lymph node regions on both sides of the diaphragm (III), which may also be accompanied by localised involvement of the spleen (III_S), extralymphatic site (III_E) or both (III_{SE}).

Stage IV: Diffuse or disseminated involvement of one or more extralymphatic organs or tissues, with or without associated lymph node enlargement.

Pathological Staging (PS)

Based on the state of involvement found at laparotomy or by any further removal of tissue for histologic examination, other than that taken for original diagnosis.

Includes annotations for specific sites biopsied:-

N+ or N- for other lymph node positive or negative by biopsy.

H+ or H- for liver positive or negative by biopsy.

S+ or S- for spleen positive or negative following splenectomy.

/...

L+ or L-	for lung positive or negative by biopsy.
M+ or M-	for bone marrow positive or negative by biopsy.
P+ or P-	for pleura or pleural fluid positive or negative by biopsy or by cytologic examination.
O+ or O-	for bone positive or negative by biopsy.
D+ or D-	for skin positive or negative by biopsy.

If the reports of the four large series mentioned earlier (Jones et al., 1973, Patchefsky et al., 1974, Brown et al., 1975, and Dumont et al., 1975) are considered together, it is possible to determine some of the typical features of the non-Hodgkin's lymphomas.

In all the series, diffuse lymphomas were more common than nodular, and the latter type were very rarely seen in patients under the age of 20. Jones et al., (1973) stated that older patients (over 60 years) also tended to have diffuse lymphomas, but the other series found no correlation in adults between age distribution and histological type. Dumont et al., (1975) found that the sex ratio of their patients was two males to one female, but this male predominance was not seen in the other series. Neither was there significant relationship

between sex and histological type, though Jones et al., (1973) stated that a greater percentage of women had nodular lymphomas and diffuse lymphomas were commoner in men. The occurrence of systemic symptoms was described in three out of the four series. Weight loss, fevers and night sweats were the commonest symptoms and were seen much more frequently in patients with Stage IV disease than in those with localised disease.

When the survival data from these series are considered, it is clear that patients with lymphocytic, well-differentiated and nodular tumours have a better prognosis for long survival than those with histiocytic, poorly-differentiated and diffuse lymphomas. Cases with widespread disease at diagnosis had a worse prognosis than cases with localised disease. The presence of systemic symptoms appeared to give an unfavourable prognosis but this was probably because they were much commoner in patients with Stage IV disease. Age at diagnosis and sex did not appear to affect the prognosis to any great extent.

Two types of initial extralymphatic involvement were frequently observed - gastro-intestinal tract and bone marrow. Once again, the prognostic significance of involvement of these sites depended on whether the tumour had a nodular or diffuse pattern, the latter giving the poorer prognosis, but according to Jones et al., (1973), gastro-intestinal tumour was commoner in patients with advanced diffuse lymphomas, and localised gastro-intestinal

involvement was rare.

Progression to a leukaemic form of the disease may occur, especially in the poorly differentiated diffuse type. Out of 323 patients studied by Stenfert Kroese, Cleton and Somers (1975), 5.5% developed leukaemia. They stated that this was not seen in patients aged between 30 and 50.

Childhood non-Hodgkin's lymphoma

The natural history of the disease in children is different from that in adults. Lingeman (1969) gave a detailed review of the literature to that date and discussed the patterns of the disease in different parts of the world. In children it is a comparatively rare tumour. Jones and Klingberg (1963) gave an incidence of 3.5 to 7.7% of childhood neoplasms. It is a fulminating disease and about 30% of cases undergo "leukaemic transformation" i.e. the disease spreads rapidly throughout the body, involving the bone marrow and occasionally the central nervous system, and leukaemia appears. They tend to have a poor response to treatment, and 50% of cases are dead within 6 months, compared with 27 months in adults. In another article by Jones and ^{13 co-authors} (1967) she stated that lymphosarcoma in children is very difficult to differentiate from acute lymphoblastic leukaemia (ALL), and recommended that the disease should have the same intensive chemotherapeutic approach as ALL. Watanabe, Sullivan, Sutow and Wilbur (1973) stated that bone marrow involvement was present in 70%

of their cases, but, surprisingly, that bone marrow and meningeal disease were not necessarily an indication of short survival. Glatstein, Kim, Donaldson, Dorfman, Gribble, Wilbur, Rosenberg and Kaplan (1974) stated that in their series of cases 97% were of diffuse lymphocytic type, and that the nodular type was rare in children. This contrasts with the figure given by Jones et al., (1973), for adults of 56% diffuse. In the study by Murphy, Frizzera and Evans (1975), 93.5% of their cases had diffuse histology. Only 7.7% of those with Stage III and IV disease survived for 3 years, whereas 50% of those with Stage I and II disease survived for that time. The gastrointestinal tract was the most common site of origin of the tumour. Central nervous system involvement occurred in 32% and leukaemia developed in 19%. In another study reported by Hutter, Favara, Nelson and Holton (1975), all cases had diffuse histology and 16 of 22 cases were diffuse poorly-differentiated lymphocytic. Males were more commonly affected than females, and this was also stated by Pinkel, Johnson and Aur (1975). Of their patients, 41 out of 64 had diffuse, undifferentiated (lymphoblastic) lymphoma. They tended to be boys under 10 years old and their median survival was 1 year. The next most common type was diffuse, poorly-differentiated histiocytic lymphoma, also commonest in boys.

The Ann Arbor staging classification has been applied and accepted as being useful in the management of the

disease. However, Lemerle, Gerard-Marchant, Sarrazin, Sancho, Tchernia, Flamant, Lemerle and Schweisguth (1973) suggested that the Ann Arbor classification was far from adequate for staging lymphosarcoma in children. Recently, the problem was discussed by Murphy (1977). She quoted an alternative staging scheme for children.

Pinkel et al., (1975) and Murphy (1977) questioned the value of the Rappaport classification in children, since most cases are of the diffuse type, and they stressed the difficulty of distinguishing between non-Hodgkin's lymphoma with bone marrow involvement and tumour cells in the blood, and acute leukaemia with extra medullary disease at diagnosis. Pinkel et al., (1975) recommended that, unless they had localised Stage I disease, patients should all be treated with anti-leukaemic therapy including multiple drug therapy, and central nervous system irradiation to prevent meningeal involvement. However, Murphy (1977), though advocating anti-leukaemic drug therapy, did not agree that all patients should have prophylactic treatment of the central nervous system.

In general, survival time in patients of all ages with non-Hodgkin's lymphoma may vary from a few months to several years depending on the factors mentioned previously, but apart from histological type, the most important prognostic sign in cases of non-Hodgkin's lymphoma is their response to treatment. The disease may be treated by surgery and/or radiotherapy if localised, but most cases, especially if widespread in the body are treated with drugs.

The best prognosis is indicated when there is a rapid response to treatment, with no signs of tumour at post-therapy staging (Skarin, Pinkus, Myerowitz, Bishop and Moloney 1974 and Lister, Cullen, Beard, Brearley, Whitehouse, Wrigley, Stansfield, Sutcliffe, Malpas and Crowther, 1978) and it is now possible that many cases may actually be cured (McKelvey and Moon, 1977). However, for those cases not achieving complete remission, for those who relapse early, and for children with fulminating disease, hope for the future must be in continuing research into new drugs and more effective combinations of existing drugs.

Aetiology

There have been several theories advanced as to the aetiology of haematopoietic neoplasms.

Lymphomas and leukaemias are known to result from exposure to ionising radiation - for example there is a high incidence of lymphomas as well as leukaemias among the survivors of the atomic bomb which devastated Hiroshima (Anderson and Ishida 1964). Apart from such obvious causes however, the theories are difficult to prove.

One theory is that lymphomas result from prolonged lymphocyte stimulation, possibly due to a failure of the feedback mechanism which "switches off" lymphocyte response to an antigen (Isliker, Leuchtenberger, and Kliem 1973). This could certainly be the reason why renal transplant recipients have a high incidence of cancers, which are often poorly differentiated lymphocytic lymphomas. However, these patients are also immunosuppressed by drugs. Untreated cancer patients especially those with advanced disease, frequently show immunosuppression and these facts lend support to another theory, the "immunologic surveillance" theory, which suggests that the tumour is due to a failure of the immune system to recognise and destroy defective cells, which are being produced all the time due to genetic errors. These theories were discussed by Schwartz (1975). Both these explanations are possible in the case of tumours induced by oncogenic viruses. Many viruses are known to cause immunosuppression. It is possible also for the virus to become incorporated

into the genome of the host cell and thus alter the growth control of the cell. Viruses are known to cause lymphomas in several species but there is little evidence as yet incriminating viruses in human lymphomas, apart from Burkitt's lymphoma in Africa, a disease mainly associated with of children. This has been shown to be due to a DNA herpes virus called Epstein Barr Virus, spread by mosquitoes (Epstein, 1970). Evidence for the presence of tumour viruses in human lymphoid tumours has been discussed by Spiegelman, Kufe, Hehlmann and Peters (1973) and Chezzi, Dettori, Manzari, Agliano and Sanna (1976).

Genetic causes or predisposition cannot be discounted when considering the aetiology of tumours. Although evidence for this is scarce in humans, there are reports in the literature of lymphomas in families e.g. Johnson and Peters (1957). Freeman, Sinks and Cohen (1970) described lymphosarcomas in siblings, associated with cytogenetic abnormalities, immune deficiency and abnormal erythropoiesis, and Maurer, Gotoff, Allen and Bolan (1976) reported malignant lymphomas of the small intestine in multiple family members, associated with an immunological deficiency. Familial Hodgkin's disease (Grufferman, Cole, Smith and Lukes, 1977) and leukaemias (Heath and Moloney, 1965, Miller 1968, Rigby, Pratt, Rosenhof and Lemon 1968 and McPhedran, Heath and Lee, 1969) have been reported.

Lymphomas in Animals

Haematopoietic neoplasms also occur commonly in animals. Lymphomas are seen much more frequently than leukaemias and have been reported in all the domesticated species, in wild animals and in fish. Disease in animals unlike that in humans, has usually been identified at post mortem examination rather than in life. Probably this is the reason why there has been a different approach to the classification of lymphoid tumours in animals. The recognised types are based on the distribution of the tumour in the body. These are:-

- 1) Multicentric
- 2) Alimentary
- 3) Thymic
- 4) Other forms, such as liver, kidney and cutaneous

Confusion over terminology has led to the use of many different names for the condition in the veterinary literature, "leucosis", "lymphomatosis", "malignant lymphoma", "malignant reticulosis", "pseudo-Hodgkin's disease", "a-leukaemic leukaemia" and other terms have all been used in the past, but the term which is now most widely used is lymphosarcoma.

Multicentric lymphosarcoma in animals has been defined by Jarrett and Mackay (1974) as follows, "A disseminated neoplasm of the lymphoid organs in which there

is usually bilateral and more or less symmetrical involvement of the lymph nodes; some regional groups may be more obviously affected than others. Involvement of the spleen is usual but may range from a mere prominence of Malpighian bodies to a marked and diffuse splenomegaly. The other organs most commonly affected are the liver, kidneys, lungs, heart, gastro-intestinal tract and bone marrow. "

Alimentary, thymic and other forms are much less common.

The alimentary type involves the Peyer's patches of the gastro-intestinal tract and associated nodes. Occasionally other organs are infiltrated.

The thymic type may involve the thymus only, or it may spread to the mediastinal lymph nodes and sometimes other organs.

The disease may involve the kidneys more than other organs - this occurs occasionally in the cat. The cutaneous form is rare, occurring chiefly in cattle and dogs.

Lymphosarcoma in Cattle

The disease in cattle may be sporadic or infectious. The latter is due to an oncogenic virus, Bovine Leukaemia Virus, (BLV), and is usually called the enzootic form.

The Sporadic Form

There is usually only a single case in a herd.

This form of the disease occurs in all ages, but most cases are calves and yearlings, and it may even be found in the newborn.

Calves less than 6 months old tend to develop the multicentric type. There is sudden enlargement of all the lymph nodes, and the superficial nodes are usually visible as well as palpable, as painless hard swellings.

Yearlings and young cattle up to the age of 30 months commonly show the thymic type, which is recognisable by a large swelling in the posterior ventral part of the neck.

In adults, there may be enlargement of the peripheral lymph nodes, but it is in older animals that the cutaneous type may be found. Plaques appear in the skin, covered with white scales, and the hair in these areas is lost. The plaques then shrink and the hair regrows. The peripheral nodes also shrink at this time, but relapse occurs 1 to 2 years later, with involvement of the internal nodes and organs.

Progressive loss of condition, depression, weakness and anaemia are clinical signs which are typical with all forms of the disease. In addition, pressure from enlarged nodes or thymus, or infiltration of organs such as the heart, liver, abomasum or central nervous system may produce a variety of other clinical signs. The temperature is usually normal unless the tumour is growing very rapidly or there is secondary infection present.

The Enzootic Form

The virus form of the disease occurs in most parts of the world and has been studied mainly in Scandinavia, Germany and North America where it is a serious problem, particularly in dairy herds. Until recently, there was no evidence that the virus form occurred in Great Britain, though the sporadic form is seen quite frequently. The essential features of the enzootic form were summarised by Anderson and Jarrett (1968) as follows:-

- (a) Several cases occur within one herd.
- (b) Most affected animals are over 4 years old.
- (c) The anatomical type is nearly always multicentric.
- (d) Apparently normal cattle in the same herd may show a lymphocytosis on haematological examination.

Anderson and Jarrett (1968) used these criteria when considering the results of their British abattoir of bovine neoplasms. survey/ The abattoirs studied killed 1.3×10^6 cattle in 1 year. From these animals, 302 specimens of bovine neoplasms were recovered and 75 (25%) of these were lymphosarcoma, the incidence being 58 per million animals slaughtered. The percentage of animals under ~~four~~ 4 years of age was 61, and the disease was found in neonatal calves on several occasions. The thymic type was more common than multicentric. Follow-up investigations among live animals did not reveal any cases,

and blood samples showed no evidence of lymphocytosis. They therefore concluded that the virus type was unlikely to occur in Great Britain. They made the point, however, that many cases would be diagnosed in life, never reaching an abattoir and estimated a true morbidity rate of 200 per million based on experience at Glasgow Veterinary Hospital.

The infectious form of the disease, known as enzootic bovine leucosis,^(EBL) has been known to be common in Germany for many years, since lymphosarcoma was first reported in cattle by Bollinger (1874). Two decades ago in certain areas of Sweden, the condition was found in 1% of slaughtered cattle (Olson, 1961). At around the same time, parts of Denmark had 10 times more cases than other areas and the disease was increasing. This situation led to the identification of multiple incidence herds, and there was evidence of horizontal transmission (Bendixen, 1960). The disease had previously been shown to be transmissible in detailed studies by Götze Rosenberger and Ziegenhagen (1956).

Investigations into the situation in the United States as well as detailed descriptions of clinical, pathological and haematological findings were published by Marshak, Coriell, Lawrence, Groshaw, Schryver, Attera and Nichols (1962) and Theilen, Dungworth, Lengyel and Rosenblatt (1964).

Measures for the control of the disease were introduced in countries where the disease was a problem.

Bendixen (1963 and 1965) described the system instituted in Denmark, which consisted of compulsory reporting of cases, haematological examinations and slaughtering out. A large scale control programme was also undertaken in Germany, and control measures, with recording of cattle introduced, are now applied in some other countries, but not U.S.A. or Canada.

Although it had long been suspected that a virus was involved, it was not proven until Miller, Miller, Olson and Gillette (1969) demonstrated Bovine Leucosis Virus in stimulated lymphocyte cultures. This was shown to be capable of producing lymphosarcoma when inoculated into calves (Miller, Miller and Olson, 1972).

Bendixen (1965) summarised reports by authors who had carried out pedigree studies in herds with the enzootic form and established a higher incidence in some cow families. The disease is known to be transmitted from cow to calf, through the placenta. However, the viral genetic material is not incorporated into the chromosomes of the reproductive cells of the host, therefore hereditary transmission does not occur, (Callahan, Lieber, Todaro, Graves and Ferrer, 1976). The main problem in the control of the disease, however, is the very long incubation period, because by the time clinical disease has appeared, widespread infection is inevitable. The exact mode of horizontal transmission has not yet been elucidated, although spread by biting insects is a possibility, and veterinary surgeons may

also unwittingly spread the disease by blood-sampling or vaccination.

A range of serological tests is now being developed, the most widely used of which is an agar gel immunodiffusion test. These tests have been reviewed by Mussgay and Kaaden (1978). The immunodiffusion test has been adopted by the EEC for control of the disease, and removal of reactor animals is now practised in some countries, as it is preferable to total herd slaughter where there is a high incidence of infection. Tyler (1978) reviewed the literature concerning the disease and the problems associated with its control.

On December 1st 1977, bovine lymphosarcoma became a notifiable disease in Great Britain. Recently, antibodies to BLV were detected in imported Canadian Holstein cattle and in in-contact animals, therefore it must be assumed that EBL is now established in the cattle population of this country (Tyler, 1978).

Lymphosarcoma in Sheep

The incidence of lymphosarcoma in sheep slaughtered in abattoirs in different countries was summarised by Bostock and Owen (1973). In most countries, the incidence of lymphosarcoma is less than 25 cases per million, and in Britain, according to Anderson and Jarrett (1968), it is only 10 cases per million, these cases being isolated and sporadic. Multiple incidence flocks were reported from Germany by Enke, Jungnitz and Rossgen (1961) and Fritzsche (1970) and in Rhodesia by Boyt, Ulbrich, Best, Paulsen, Nitzschke /

Mackenzie and Emslie (1976). Lymphosarcoma is one of the commonest tumours of sheep in most countries but is a relatively uncommon tumour in New Zealand according to Cordes and Shortridge (1971). These authors' survey also gave different results from those of other authors about the age of animals affected. Fourteen of their 20 cases were less than 1 year old whereas Webster (1967), also in New Zealand, Anderson and Jarrett (1968) and Migaki (1969) agreed that it occurs mostly in older animals.

Clinical reports in the literature are rare since most cases are diagnosed at autopsy. Nobel, Neumann and Klopfer (1967) described one animal with jaundice as the presenting sign, and another with subcutaneous nodules. The clinical and haematological findings in one of the multiple incidence flocks were described by Ulbrich et al., (1970). Enlarged submaxillary nodes and pneumonia were the most obvious signs.

Multicentric lymphosarcoma is the most common anatomical type but thymic and skin types are seen.

Bovine Leucosis Virus has been shown to be capable of causing lymphosarcoma in sheep (Olson, Miller, Miller and Hoss, 1972 and Van der Maaten and Miller 1976).

Lymphosarcoma in Horses

Many authors have credited Leisering with the first published report of lymphosarcoma in an animal in 1853.

This was a description of the disease in a horse. The literature has been reviewed by Jarmai (1934), Cotchin (1956), Dekker and Kroneman (1958), Squire (1964), Misdorp (1966) and Lamberg (1968). Neufeld (1973a) cited 91 cases reported in the literature during a period of 120 years. He subsequently (1973b) reported another case.

Lymphosarcoma in the horse apparently occurs in all parts of the world. Four to nine years old is the age group with most cases although the disease has been reported at all ages except in horses over 23 years. All anatomical types are seen. However, single circumscribed tumour masses seem to be commonly mentioned in post-mortem findings particularly in males. The mare appears to be more likely to have widespread tumour involvement of lymph nodes and other organs. Clinical signs vary considerably, and differential diagnosis is not always easy when the animal is presented with signs such as loss of condition, cough, abdominal enlargement, attacks of colic or shifting lameness.

Haematological findings also vary, though relatively few authors have studied this aspect in detail. Neufeld summarised findings in twenty six cases in which 15% were leukopenic, 58.3% were leukaemic and 36% were anaemic.

It is likely that many cases of equine lymphosarcoma may not be diagnosed. Nevertheless it is quite a rare condition. Cotchin (1960) saw 90 horse tumours - only

two were lymphosarcoma and were classified as alimentary. Smith (1962) quoted an incidence in slaughtered horses of 1.55 per 100,000. Migaki (1969) quoted figures taken from the records of the Federal Meat Inspection service (1957 - 1967) which show wide variation from year to year (because of irregular culling practices) but the figures are still low when one considers that the horses slaughtered are usually adults (0 - 18.75/100,000).

Genetic susceptibility has not been investigated, and there is only one report which suggests possible viral aetiology (McKercher, Wada, Straub and Theilen 1963).

Lymphosarcoma in Cats

Haematopoietic tumours are among the commonest malignancies of the cat. (Cotchin, 1952 and 1956^a, and Holzworth, 1960). In 1964, the study of lymphosarcoma in the cat was dramatically directed along new lines by Jarrett's investigation of a cluster of cases in unrelated cats in one household in Glasgow. This led to successful transmission of the disease to susceptible kittens and to the isolation of Feline Leukaemia Virus. (Jarrett, Crawford, Martin and Davie 1964a and b).

Feline Leukaemia Virus (FeLV) is an RNA virus of the retro-virus group. The epidemiology of the condition has been investigated (Hardy, Old, ^{and Cotton} Hess, Essex, 1973) and it has been shown that the virus can be spread horizontally, probably shed by infected cats in urine and

saliva, and from dam to offspring in utero and via the milk. It is much commoner in cities than in country areas. It may be a serious problem in breeding colonies if infected cats are introduced.

The fate of an individual cat which becomes infected depends on the type and dose of virus and the ability of the cat to mount an immune response against the three classes of antigen associated with the virus. Two types of antibody have been identified - virus neutralising antibody and antibody against feline oncornavirus - associated cell membrane antigen (FOCMA).

The conditions produced by FeLV have been described by Mackey (1975). The virus affects rapidly dividing cells and produces both degenerative and proliferative diseases. It is associated with 90% of cases of lymphosarcoma in cats (Hardy and McClelland, 1977). It also causes leukaemias, both lymphoid and myeloid, and it may cause malignancies of red cell, monocyte or thrombocyte precursors.

Anaemia is often seen in FeLV infected cats. This may be associated with lymphosarcoma or leukaemia, or it may not. If not it may be haemolytic or aplastic. FeLV also causes immunosuppression and thymus atrophy in kittens (Anderson, Jarrett^{Jarrett} and Laird 1971) and has been implicated in cases of abortion, foetal resorption and neonatal deaths (Cotter, Hardy and Essex, 1975).

There is an association between FeLV infection and feline infectious peritonitis, also glomerulonephritis

and a panleucopenia-like syndrome.

A simple immunofluorescence test has been developed by Hardy, Hurshaut and Hess (1973) in the United States for the detection of FeLV antigens and this is being used in the control of the disease.

Useful descriptions of feline leukaemia and the effects of the virus have been published by Jarrett (1971) Jarrett, Mackay, Jarrett and Laird (1973) and Hardy and McClelland (1977).

Lymphosarcoma in Dogs

Lymphomas are common neoplasms in dogs. According to Cohen, Booth and Sussman (1959) based on reports by several authors, these neoplasms have an incidence of 0.1% to 0.2% in all dogs, account for 1 to 4% of canine tumours. MacEwen, Patnaik and Wilkins (1977) reported a figure of 5-7% of canine tumours. Jarrett, Crighton and Dalton (1966) found that lymphosarcoma constituted 22% of fatal malignant neoplasms in the dog which is much higher than the 8-9% given by Dorn, Taylor and Hibbard (1967).

There is still no real evidence of viral aetiology, though the disease can be transplanted experimentally. This was achieved by Moldovanu, Moore, Friedman and Miller (1966), by Owen and Neilsen (1968) and Kakuk, Hinz, Langham and Conner (1968), using Beagle puppies as recipients of transplanted cells.

The sporadic disease is usually multicentric or alimentary, though the other types are seen, and is more

common in older dogs. Some breeds of dog seem to be more susceptible than others. Breeds commonly mentioned are the Boxer (Dorn et al., 1967, Priester, 1967, Squire 1969) the Scottish Terrier (Bloom and Meyer 1945, Jennings 1953, and Cotchin 1956**b**) and the Cocker Spaniel (Squire 1969, MacEwen et al., 1977).

The literature on lymphosarcoma in the dog is very extensive but was reviewed by Jarmai (1934), Bloom and Meyer (1945) and Cotchin (1956**b**). The clinical, haematological and diagnostic aspects of the disease were discussed by Bloom and Meyer (1945), Jennings (1953), Squire (1964 and 1969), Jarrett et al., (1966), Van Pelt and Conner (1968) and MacEwen et al., (1977).

Clinical signs vary, depending on the extent and location of the disease. Sometimes the dog is presented with non-specific signs such as fever, weight loss and anorexia. Most dogs with multicentric lymphosarcoma have painless enlargement of the superficial lymph nodes, and the tonsils may also be enlarged. Dogs with the alimentary form usually have vomiting and diarrhoea. Leukaemia is usually absent except in the very late stages. A neutrophilia is often discovered and the dog may be anaemic.

Lymphoid Tumours in Laboratory Animals

Mice

Leukaemias and lymphomas are rare in wild mice (Gardner, Henderson, Rongey, Estes and Huebner 1973) but are very common in laboratory mice. Strains of mice with a high incidence of

spontaneous leukaemia have appeared during inbreeding, sometimes with selection for this character, sometimes in the course of selection for other characters. There are also strains with low or intermediate incidence of leukaemia. The inheritance of a liability to develop leukaemia is probably multifactorial and its investigation by standard Mendelian methods has not been very successful. The genetic factors may include rate of cellular differentiation, hormone balance and cellular susceptibility to infection and malignant transformation by viruses (Salaman, 1967).

A murine leukaemia virus was first discovered by Gross (1951). He reported that leukaemia could be induced in certain inbred strains of mice by the inoculation of cell-free extracts of liver, spleen and mesenteric tumours from mice with spontaneous or transplanted leukaemia.

Following this, some 14 different leukaemogenic RNA murine viruses have been isolated (Friend 1957, Moloney, 1960, Rauscher, 1962). Viruses from high leukaemic strains, when injected into suckling mice, induce leukaemia in significant incidence in low-leukaemic strains and accelerate its appearance in the parent strain. The infant mouse is much more susceptible to injected leukaemogenic viruses than the adult, probably because there is a scarcity of mature lymphocytes and an abundance of relatively undifferentiated lymphoid cells in the cortex of the thymus.

Leukaemogenic viruses are remarkable for the variety of effects they produce, and yet different viruses may produce diseases which are pathologically indistinguishable.

Many laboratory strains of murine leukaemia viruses probably do not bear any aetiological relationship to the tumour from which they were derived e.g. Moloney strain, which causes lymphocytic leukaemia, was derived from sarcoma 37, and Friend's leukaemia virus originated from an adult mouse inoculated shortly after birth with a cell-free extract of Erlich ascites tumour (Buxton and Fraser, 1977).

Transplantable tumours are important for research purposes because they provide predictable systems. Two well-known examples are L1210 and P388 leukaemias, and these are routinely used for screening of new anti-cancer agents.

It is very difficult to find a strain of laboratory mice entirely free from leukaemia, and even in mice of strains known to have a low incidence of spontaneous tumours, it is possible that lymphosarcoma or leukaemia can be induced by total body irradiation or by administration of hormones or chemical carcinogens.

The murine leukaemias have been repeatedly reviewed, e.g. by Furth (1951), Kirchbaum (1951), Law (1954), Miller (1961) and Moloney (1964).

Rats

Most murine leukaemia viruses produce leukaemia in rats. Spontaneous lymphosarcoma and leukaemia, however, are quite rare in rats (Bullock and Curtis, 1930, McEuen 1939, Kim, Clifton and Furth, 1960).

In 1957, Dunning and Curtis reported a transplantable

acute leukaemia in in-bred rats, and this is still used for experimental purposes at the National Cancer Institute. (Goldin, Venditti and Carter, 1977).

Guinea-Pigs

Leukaemia is rare in guinea-pigs, but there is a virus-induced type (Opler, 1969).

Hamsters

Mouse Moloney virus produces leukaemia in hamsters (Buxton and Fraser, 1977).

Rabbits

Lymphosarcoma is uncommon in the rabbit and a virus has not yet been implicated. Kampen (1968) reviewed the literature, as well as describing a case.

A hereditary form of lymphosarcoma in WH strain rabbits was reported by Fox, Meier, Crary, Myers, Norberg and Laird (1970). The condition is apparently inherited in association with an autosomal recessive gene.

Affected rabbits were usually less than 1 year old when the disease was diagnosed. Clinical signs were weight loss, lethargy and anaemic mucous membranes. High white blood cell counts were infrequent but there was a predominance of lymphoid cells in smears and many appeared to be blast cells.

At autopsy, visceral lymph nodes were enlarged and spleen, liver and kidneys were usually involved. The thymus and adrenals were enlarged in some rabbits.

A few of the cases had produced progeny, but it

appears that an affected rabbit was never mated to another affected rabbit to produce 100% affected progeny. Nevertheless, the ratios of affected to normal rabbits obtained from other types of mating suggested a Mendelian type of inheritance.

Lymphoid Tumours in Poultry

The Avian Leukosis Complex (Helmboldt and Fredrickson, 1969).

The avian leukosis complex is a group of viral diseases which results in major economic losses in the poultry industry. Most diseases of the complex are disseminated proliferative processes involving cells of the haematopoietic system or their precursors. The most prevalent are those involving lymphoid cells.

The causative viruses of most avian solid tumours are related to those causing disseminated haematopoietic diseases. Both RNA and DNA viruses have been implicated.

The two most important diseases are lymphoid leukosis, also called lymphomatosis, and Marek's disease. Unlike in man and ^{other} mammals, spontaneous occurrence of these diseases reaches epizootic proportions and in some areas are considered to be enzootic.

Lymphoid leukosis

Ellermann and Bang (1908) first reported transmission of lymphoid leukosis by cell-free filtrates. It is a disease of mature birds. The liver and spleen are most often involved, though any organ may be affected. Bone marrow is commonly involved but tumour in the brain

is rare. The lymphomas are usually multiple and the affected organs are usually enlarged.

The virus first attacks the cells of the follicles of the Bursa of Fabricius. If the bursa is removed at the time of hatching, the disease fails to develop, (Peterson, Burmester, Fredrickson and Good 1963). A tumour is formed in the bursa, which metastasizes primarily to the liver and spleen. Leukaemia has been reported but is rare. Both vertical transmission of virus through the egg and horizontal transmission among chicks occur, but the carrier hen is the chief vehicle. Elimination of carrier hens can prevent transmission of the disease.

Marek's Disease

This disease is also called neurolymphomatosis or fowl paralysis. It was first described by Marek (1907) as a polyneuritis, but it has many features of a neoplastic process. The disease is seen in growing birds and is characterised by multiple neoplasms of lymphoid cells that are difficult to differentiate from those of lymphoid leukaemia. The central nervous system is the site of a diffuse non-purulent encephalomyeloradiculitis. This rarely progresses to become neoplastic. In peripheral nerves, an inflammatory lesion with oedema and a diffuse infiltration of lymphocytes, histiocytes and plasma cells is believed to precede the formation of diffuse lymphomas. The sciatic nerve and dorsal root ganglia are common sites. Gross visible enlargement of

the nerves may be seen and there may be clinical paralysis. Lymphomas may be present in viscera and muscles, but neoplastic proliferations are not seen in all birds. Some birds apparently recover from the disease.

In the acute form of the disease, the ovaries, lungs and haematopoietic tissue, predominantly the bone marrow, are overrun by lymphoblastic cells, resulting in lymphosarcoma. The bird usually dies before obvious anaemia or leukaemia develops.

In 1967, electron microscopic examination of blood lymphocytes and cell cultures infected with cells from birds with Marek's disease showed particles resembling DNA virus of the Herpes group (Biggs, 1967, Wight, Wilson, Campbell and Fraser, 1967, Nazerian, Solomon, Witter and Burmester, 1968).

It was shown by Churchill, Payne and Chubb (1969) that immunization of birds against Marek's disease was possible, using a live attenuated strain of virus, and because of the major commercial importance of the disease, vaccination in poultry flocks is now carried out.

Other viral neoplastic diseases are erythroblastosis, myeloblastosis, myelocytomatosis and various solid tumours.

The classification of diseases induced by the avian tumour viruses has been frequently revised, (Campbell (1961), Biggs and Payne (1967), Beard, Langlois and Beard (1973) and others.

Lymphosarcoma in Pigs

As in some other species already considered, lymphosarcoma is not rare in swine. It is the most common pig tumour according to Plummer (1956), Cotchin (1960) Jarrett et al., (1966) and Fisher and Olander (1978). Many cases have been reported in the literature and, as in cattle, records of the incidence of the disease are available from abattoir surveys. Bostock and Owen (1973) have summarised the incidence in slaughtered pigs in reports from Belgium, France, Germany, Great Britain, Netherlands, United States and Norway. In all these countries, except France, the incidence is under 25 cases per million animals slaughtered, though probably many never reach slaughter weight. In Brittany however, the incidence is given by Renier, Friedmann, Chevrel, Gacquiére and Guelfi (1966) as 65 per million overall, the incidence in the eastern area of the province being six times that seen in the west. A wider survey in France by Lombard (1968) showed a very low incidence in many areas but a very high incidence in a few regions, particularly Gers in the south-west, with over 2000 cases per million. There is no suggested explanation of this extraordinarily high figure by Lombard, but Renier et al., (1966) suspected that a virus might be involved in Brittany. Anderson and Jarrett (1968) followed up every one of 92 cases diagnosed at the abattoirs in their survey in Great Britain, and in no case was there any evidence that the disease was

present in other animals, or that it had occurred before on any of the farms. The literature from other parts of the world also indicates that the disease is sporadic - isolated cases only are reported. The extensive literature has been reviewed by Jarmai (1934), Englert (1955), Cotchin (1956b), Squire (1964), Lombard (1968) and Bostock and Owen (1973).

Most authors seem to agree that the disease is commonest in young animals. The majority of Englert's (1955) cases were under one year old. Of Anderson and Jarrett's 92 cases, 59% were immature animals. In the series of 200 cases described by Migaki (1969) 60% were between 6 and 8 months of age. Of the cases seen by Renier et al., (1966) 80% were around 6 months. However, the actual prevalence of cases was proportionately lower in this age group than in the older age group (mean age 21 months) when they consider the number of animals of each age group slaughtered. In 15 cases recorded by Cotchin (1960) the age range was newborn to 16 months.

The pathological types of lymphosarcoma seen in the pig are multicentric or thymic. Of the cases recorded by Jarrett et al., (1966), 30% were thymic. Despite the large volume of literature on the subject, the vast majority of reports are essentially pathological descriptions and clinical and haematological findings are rare. As in other species gross enlargement of superficial lymph nodes may be a prominent feature of some cases of multicentric lymphosarcoma. On the whole, however, cases are

not usually diagnosed in life, and young pigs with lymphosarcoma are probably disposed of by the farmer without investigation.

Englert (1955) discussed in detail the clinical findings both as reported in the literature to that date, which consisted of only nine references, and his own experience of 130 cases, the majority of which were sent for emergency slaughter. Clinical signs were noted by him in 22 cases. Reduced appetite and loss of condition were the main signs but constipation, an impression of exhaustion, laboured respiration, fever, recumbency, hind-leg lameness, difficulty in swallowing and haemorrhages of the skin were also seen.

Reichel (1962) described two cases in detail. Clinical findings, results of blood serum and bone-marrow examinations were recorded, and the terminal course of the disease was monitored. Both pigs were leukaemic especially Pig II which had a white blood cell count of 91×10^9 /litre. Photographs showed that the superficial lymph nodes were very large. Pig I was treated with prednisolone for three days, but it died despite the fact that the blood lymphocyte and blast counts fell. Adamesteanu and Adamesteanu (1969) reported three cases of lymphosarcoma, one of which was diagnosed in life. The clinical course was acute with a white-blood cell count of 200×10^9 /litre. The pathology was described in detail.

Where figures are quoted from references, they have been altered to S.I. units.

Chevrel, Renier, Richier, Ramee and Treguer (1969) reported their findings in 38 cases. The most common clinical signs were unthriftiness, anaemia (with jaundice in one case) respiratory trouble in five cases and in four cases, the presence of localised tumour in the neck region.

Stevenson and DeWitt (1973) described an unusual case in a 13 month-old boar with only loss of appetite and mild fever for three days before death occurred. The main gross pathological lesions were haemorrhages throughout the body. Invasion of most organs by immature lymphocytes was seen on histological section.

Transmission of the disease to one-day-old piglets was attempted by Case and Simon (1968). Although some lymphoid hyperplasia was seen in lymph nodes of inoculated pigs they did not develop the disease, and the lymphocytosis reported, which regressed, was probably physiological. A lymphocytosis often occurs in normal pigs at from 9 to 16 weeks of age (McTaggart, 1975).

The production of C-type virus particles by a permanent cell line obtained from tissues from a pig with lymphosarcoma was reported by Strandstrom, Veijalainen, Moennig, Hunsmann, Schwarz and Schafer (1974). These particles were shown to be capable of infecting normal pig kidney cells. In general, however, there is as yet no ^{convincing} evidence implicating viruses in the aetiology of lymphosarcoma in the pig.

Hereditary Pig Lymphosarcoma

In 1963, at the Royal (Dick) School of Veterinary Studies Field Station near Edinburgh, a new piggery was set up to investigate the possible beneficial effects on productivity of a minimal disease environment.

The foundation stock were hysterectomy derived Large White pigs, which were hand-reared. The piggery has since remained closed, and although new blood-lines have been introduced occasionally by means of artificial insemination, inevitably a degree of in-breeding has been necessary.

In order to check on minimal disease status in the piggery any animal which died was sent for autopsy. In 1966, lymphosarcoma was diagnosed at autopsy in a three-month-old pig which had been culled because of failure to thrive. The significance of this fact might have gone unappreciated, were it not for an observation by the pathologist that it was one of a litter resulting from a brother/sister mating, and that possibly a genetic factor might be involved. The rest of the litter were examined and blood samples revealed in one of them a lymphatic leukaemia. Unfortunately, the mating could not be repeated because the boar had been disposed of, and although another boar was obtained, a full brother of the sire of the piglets, no more cases were produced.

In 1969 several cases of lymphosarcoma appeared in different litters and when the pedigrees of these animals

were examined, it became obvious that they were exclusively inbred descendents of the same boar which had been the sire of the first two cases. By the end of 1974, 64 cases had been identified. These had 211 normal litter mates which survived to bacon weight at 5 to 6 months of age. These cases represented 23.27% of the litters in which they occurred. This is not significantly different from the 25% to be expected ($\chi^2 = 0.44$) if transmission of the disease is associated with a recessive gene. The condition was reported by McTaggart, Head and Laing (1970).

Further studies have defined the characteristic features of the disease. The clinical signs, haematology and pathology have been described by Head, Campbell, Imlah, Laing, Linklater and McTaggart (1974). The ultrastructure of lymphosarcomatous tissues from these pigs has been reported by Campbell (1977). The main characteristics of the disease are as follows:

- (a) The disease occurs in young pigs and can usually be diagnosed between 6 and 8 weeks of age. The longest surviving untreated case was 17 months old.
- (b) It is not sex-linked, occurring fairly equally in males and females.
- (c) No untreated case has ever reached functional sexual maturity therefore it has never been possible to confirm the genetic theory by mating two affected animals to produce 100% affected piglets.
- (d) All cases are clinically and pathologically similar except for the rate at which the disease develops

and the effects of intercurrent disease.

- (e) Carrier parents so far appear to be identical in every way to normal non-carrier animals. No genetic marker has yet been found, therefore mating with a known carrier is the only method of testing a potential carrier, at present.
- (f) The disease is not transmissible by any means yet tried, between pigs.

Clinical Features

The cardinal clinical features of the disease are:

- (a) Stunting of growth.
- (b) Moderate enlargement of superficial lymph nodes particularly inguinal and precrural nodes since these are most readily visible and palpable. Gross enlargement as sometimes seen in the sporadic form never occurs.
- (c) Pot-bellied appearance see Plate (1).
- (d) Upward arching of the spine.

Fever is unusual, except as a result of intercurrent infection. In advanced disease bodyweight levels off, rarely reaching bacon weight (90kg), and then fluctuates from week to week, probably due largely to variations in gut-fill, as the appetite becomes capricious. During spells of "natural remission" (see haematology) the appetite is good and the pigs are quite lively, but between these spells they are listless, reluctant to rise and have poor appetites. Eventually, usually during one of these periods of decline, death occurs, if the animal

has not succumbed previously as a result of intercurrent disease such as colibacillosis or necrotic enteritis. Actual causes of death are very varied but the tumour itself is rarely the direct cause unless death results from severe haemorrhage.

All the characteristic features may be seen in a variety of other conditions, and therefore haematological examination is necessary.

Haematology

The blood picture in lymphosarcoma varies considerably and often leukaemia does not appear until the later stages of the disease. Renier et al., (1966) considered that haematology was not very useful in the diagnosis of sporadic lymphosarcoma in pigs, as 10 of the 25 cases that they sampled had a normal blood picture. In hereditary porcine lymphosarcoma the following haematological features are usually seen from about 6 to 8 weeks of age onwards:-

- (a) Total white cell counts within the normal range ($10 - 20 \times 10^9$ /litre) or moderately elevated. Counts rarely exceed 30×10^9 cells/litre, though occasionally counts of up to 100×10^9 /litre are found for a short time in an individual animal. The highest white cell count observed was in a treated animal in relapse - 160×10^9 /litre.
- (b) Over 70% mononuclear cells on differential count.

- (c) Abnormal mononuclear cell morphology i.e. large cells with pale often vacuolated cytoplasm and irregular lightly staining nuclei, frequently with nuclear protrusions. Occasionally mitotic figures are seen.
- (d) A progressive macrocytic, sometimes hypochromic anaemia with very few normoblasts.
- (e) A progressive thrombocytopenia.

When diagnosis is attempted between 9 weeks and 16 weeks, difficulties are occasionally encountered because normal pigs of this age tend to have a lymphocytosis (McTaggart, 1975). High white cell counts and percentages of lymphocytes of 70 to 80 may be encountered. The morphology of the cells is quite different however, consisting of a high proportion of darkly staining, typical small lymphocytes.

If the course of the disease is followed in any one animal it is found that there are sometimes periods of natural remission when the total white cell count falls and the anaemia improves. In between, there are spells of decline as mentioned previously, when the opposite occurs and the thrombocyte count may fall to such an extent that haemorrhage may readily occur. In the terminal stages of the disease, the total white cell count and percentage of abnormal cells usually fall.

At autopsy, all cases are grossly and histologically essentially alike though the degree of abnormality seen

depends on the age at death. In the earlier years before the start of therapeutic studies, many lymphosarcoma pigs died at a fairly early age mainly due to post weaning colibacillosis. In life some showed advanced disease at this time, others appeared relatively normal apart from enlarged nodes and white blood cell picture typical of the disease. Age at death showed a bimodal distribution. If the pigs did not die before 120 days old, they tended to live much longer, to a mean of 180 days and sometimes as long as 15 months.

Pathology

The most obvious gross pathological feature is the enlargement of the lymph nodes but the gastric, mesenteric and bronchial nodes show a much greater degree of abnormality than the other visceral and carcass nodes. The gastrosplenic and mesenteric nodes in the more advanced cases may be 30 times (and the bronchial nodes up to eight times) the weight of those of normal pigs of comparable age. On cut surface, a flat field of pale fawn or white tumour tissue is seen, except for the gastrosplenic which may be dark red in patches, due to haemorrhage and fibrin. The cut surface of the superficial nodes, which enlarge to approximately 1.5 times normal weight, may show the remains of normal lymph node architecture, especially in the less advanced cases. Cortical nodules are seen as well as tumour tissue and these may be surrounded by a darker coloured area of medulla.

The thymus is never involved in the tumour and in fact it is usually involuted. The spleen is enlarged - in the advanced cases it may be very large indeed - and it may have nodules on the visceral surface which on section are deeper red than the remainder of the spleen which is soft with a mottled bulging lobular pattern. Splenic infarcts are occasionally seen, also torsion of the ventral end of the organ in a few cases.

The tonsils are not enlarged. The glandular mucosa of the stomach may have numerous white nodules. The Peyer's patches of the small intestine are prominent and nodules are seen in the wall of the caecum and colon. The liver enlarges progressively with the stage of the disease and becomes paler in colour. Occasionally, focal areas of tumour infiltration are seen or even white nodules of tumour tissue. Gross tumour involvement of the epididymis often occurs but the ovary and uterus are never affected. Occasionally tumour nodules may be seen in the kidneys.

The marrow of the diaphysis of long bones (the femur is usually examined) may contain white foci of tumour tissue. It is usually red, and active with depletion of fat in most cases.

Histopathology

On histological examination, the white tissue seen macroscopically appears as large numbers of lymphocytes, many of them typical large tumour cells with mitotic figures. They are found in the perilobular connective

tissue of the liver, peribronchiolar tissue in the lungs, in the red pulp of the spleen, in perivascular cuffs at the cortico medullary junction in the kidney, in the interstitial tissue of the testis and peritubular connective tissue of the epididymis, in the submucosa of the intestine and in the bone marrow.

Other common pathological findings in lymphosarcoma pigs are gastric ulcers. These are usually in the oesophageal region but may also be found in the fundus. Haemorrhage from these ulcers is fairly common and occasionally they perforate, causing escape of stomach contents into the peritoneal cavity and death from acute peritonitis.

Hydrothorax, hydropericardium and ascites may also be found especially in those which have died. (It is not usually present in animals which are deliberately destroyed, but has been found in fairly advanced cases at laparotomy - see later.)

Widespread petechial haemorrhages may be observed and animals which have been bullied by their normal litter mates often have severe subcutaneous and internal haemorrhages.

The possibility of viral aetiology has been investigated, and reverse transcriptase has been detected. This enzyme is necessary for the incorporation of viral RNA into the DNA of the host cell. C-type virus particles have been seen occasionally in ultrastructural photographs of tumour cells. However, if a virus is

involved it is undoubtedly completely incorporated into the DNA of the host, even within the reproductive cells, and is unlikely to be released as free virus. The fact that the disease cannot be transmitted would appear to support this theory.

Chemotherapy

Cancer chemotherapy means treatment of cancer with drugs. The rationale of anticancer drug usage is to selectively destroy or control the growth of malignant cells. Malignant tissue is made up of dividing cells and nondividing cells, the relative proportions of each depending on the type of tumour. Generally the larger the tumour, the slower is the rate of cell division. Dividing cells go through a series of events which has become known as "the cell cycle". Most anti-neoplastic drugs interfere with nucleic acid production and function.

The phases of the life cycle of the dividing cell are:-

- (1) Mitosis (usually given the letter "M").
division)
- (2) Gap one (G_1) a relatively quiescent phase during which RNA and protein synthesis occurs.
- (3) DNA synthesis (S-phase).
- (4) Gap two (G_2) another apparently resting phase during which there is more RNA and protein synthesis and construction of the mitotic apparatus.
- (5) Mitosis.

The S, G₂ and D phases in most dividing cells are fairly constant. The variation in length of the cell cycle occurs during G₁ phase. When proliferative activity is high, G₁ may be very short. When it is low, G₁ may be so long that the cell seems to become dormant, ceasing to divide. It is then described as being in G₀ phase.

After a variable time, these cells may resume division and return to the cell cycle.

Different drugs act at different phases of the cell cycle. Some drugs may kill cells at any stage in the cycle. These are classed as phase non-specific. Most drugs, however, act at one stage in the cycle and are classed as phase specific.

Replicating cells are most sensitive to attack usually during S phase. Those in G₀ are very resistant, making the task of destroying every tumour cell in the body a very difficult one.

Although drugs may be classified as phase specific or non-specific, the most usual classification is based on their mechanisms of action. These are as follows:-

- 1) Hormones - Steroids
 - a) Androgens,
 - b) Oestrogens
 - c) Progesterone
 - d) Adrenal cortical.

The first three groups are useful because cancers from hormone - sensitive tissues may retain some of their hormonal responsiveness and their effects are exaggerations of their physiological effects. The mechanism

of action of the corticosteroids in cancer is not known but may be related to the presence of corticosteroid receptor proteins within sensitive cells leading to direct nuclear damage (See Chapter I).

2) Alkylating agents.

These are agents which can substitute an alkyl ($R - CH_2 - CH_2^+$) group for the hydrogen ions of many organic compounds. Their cytotoxic effects are thought to be due to the formation of a covalent bond between a side limb of the drug molecule and the 7 - nitrogen group of guanosine, and other sites may also be attacked. Since most alkylating agents have more than one side chain, "cross-linking" of DNA occurs and breaks are produced in the molecule, preventing division of the helix in mitosis or if it does divide, causing it to reunite in an imperfect form. These effects result in cell death. As similar effects are produced by certain kinds of ionising radiation, these drugs may be described as "radiomimetic".

3) Antimetabolites

These are structural analogues of naturally-occurring compounds, capable of entering into the series of metabolic reactions leading to the synthesis of nucleic acids. They react with a specific enzyme, causing inhibition of the enzyme, or synthesis of a molecule which is incapable of functioning normally.

4) Anti tumour antibiotics

Like the antibacterials, these are natural products of fungi and particularly the genus Streptomyces. Their mechanism of action is to form a stable complex with DNA, thereby inhibiting synthesis of DNA or RNA or both.

5) Unclassified agents e.g. the Vinca Alkaloids.

These drugs have large and complex molecules, and are extracted from the periwinkle plant (Vinca rosea). They bind to the essential contractile proteins of the mitotic spindle of dividing cells and this leads to mitotic arrest.

Cancer chemotherapy is one of the most rapidly developing fields in medical science, most advances having been made since the 1950s.

However, according to Haddow (1970) the chemotherapeutic approach to cancer is very old indeed - at least 1500 years. In Renaissance times, there was much interest in it and most of the drugs used contained inorganic elements such as arsenic. In more recent times, potassium arsenite was used by Lissauer (1865) to treat leukaemia. Coley (1893) attempted to treat malignant disease by repeated injections of ^{cultural material of human} erysipelas. Lead salts were also used in the early 1900s.

After this, it seems there were no real developments until the 1940s when the chemotherapy of bacterial disease was advancing rapidly. The steroid hormones were found to have anti tumour effects. Oestrogens were used in carcinoma of the prostate and oestrogens or

androgens in breast carcinoma.

Heilman and Kendall (1944) demonstrated the effect of cortisone in mouse lymphosarcoma and by 1949 ACTH and cortisone were shown to be effective in lymphoid tumours in man. (Pearson, Eliel, Rawson, Dobriner and Rhoads 1949). The first alkylating agent was also introduced in the 1940s. This was nitrogen mustard, which had been developed as a clinical weapon in World War I. Krumbhaar and Krumbhaar (1919) studied soldiers dying of bone marrow aplasia as a result of being exposed to mustard gas, and during World War II it was shown to cause damage to lymphoid organs as well as bone marrow. This led to trials first in mouse lymphosarcoma, and then in patients with lymphoid tumours (Gilman, 1963). Since then, many improved derivatives of nitrogen mustard have been developed, such as cyclophosphamide, chlorambucil and melphalan.

By the 1930s the sulphonamides had been shown to be active against various bacteria because of a metabolite - antimetabolite relationship with para-amino benzoic acid. This theory of antimetabolites led to the synthesis by Seeger, Smith and Hultquist (1947) of the folic acid antagonists aminopterin and amethopterin (methotrexate). Farber, Diamond, Mercer, Sylvester and Wolff (1948) showed that aminopterin could regularly produce remissions in acute leukaemia in childhood. By 1955 a range of antagonists of purines and pyrimidines (such as mercaptopurine and thioguanine) had



been synthesised, based on the studies by Hitchings, Elion, Falco, Russell and Vander Werff (1950). In 1957, Heidelberger, Chanduri, Danneberg, Mooren, Griesbach, Duschinsky, Schnitzer, Plevin and Scheiner, reported the synthesis of 5-fluorouracil, an antimetabolite of uracil which has proved extremely useful in adenocarcinoma of the colon, stomach, pancreas and breast. A different type of pyrimidine antimetabolite was synthesised in 1959, namely cytosine arabinoside (ara-C). This was shown to be active in mouse leukaemias by Evans, Musser, Bostwick and Mengel (1964). This compound has now become important in treatment of leukaemias in man, particularly acute myeloid leukaemia (Ellison, and 20 co-authors 1968).

The first cytotoxic antibiotics were the actinomycins which were discovered by Waksman and Woodruff (1940). Because of the severe toxicity of these compounds there was little interest in them until 1952 when they were shown to be active in mouse tumours by Hackman (1952), and also in Hodgkin's disease by Schulte (1952). This led to the development of many more antibiotics with anticancer activity, the most effective of which are mitomycin, bleomycin, which is unusual because it has no bone marrow suppressant effect, and daunorubicin and its now widely used derivative doxorubicin (adriamycin). This last drug was introduced in 1969 (DiMarco, Gaetani and Scarpinato 1969).

In the late 1950s, Johnson, Armstrong, Gorman and Burnett (1963), and others, demonstrated that an extract of the periwinkle Vinca rosea was active against leukaemia in the mouse. The compounds vinblastine and vincristine

were isolated and were shown to be effective in the treatment of Hodgkin's disease, acute lymphoblastic leukaemia and other tumours (Warwick, Darte and Brown 1960, Armstrong, Dyke and Fouts 1962, Karon, Freirich and Frei 1962).

The greatest advances in cancer chemotherapy have occurred mainly since 1968 with the development of combinations of drugs which act in different ways so that the problem of additive toxicity is avoided. Initial studies reviewed by Goldin and Mantel (1957) laid the foundations for the protocols which are now so successful in the treatment of Hodgkin's disease and some of the other leukaemias and lymphomas. The principles of selection of drugs have been described by DeVita, Young and Canellos (1975).

Drug Toxicity

The major problem in cancer chemotherapy is drug toxicity. As cytotoxic drugs mainly attack cells in division, normal tissues with a naturally high turnover of cells such as bone marrow, gut, hair follicles, ovary and testis may be damaged almost to the same degree as the tumour cells.

The most serious toxicity is bone marrow depression, and this occurs with most of the drugs in common use. Marrow depression may result in neutropenia, leading to infection, or thrombocytopenia, leading to bleeding or anaemia. The immune response is generally depressed, but the effects on specific immune responses depend on the drug and how it is administered - occasionally there

is an increase in a specific immune response. This has been noted in animals as well as humans (Schwartz 1969), but there is at present no clear explanation for this. Increased susceptibility to infection is a great problem, as many of the infectious agents are opportunists which would not normally cause disease in a healthy individual.

Gastro-intestinal toxicity may result in ulcerative stomatitis, pharyngitis, vomiting and diarrhoea. Nausea is very common in man. Alopecia is a frequent side-effect of many drugs in man and some animals.

Effects on the ovary and testis usually result in sterility but if conception does occur, cytotoxic drugs are then liable to be teratogenic.

In addition to these common toxicities, antineoplastic drugs often have side effects on organs and systems which are independent of their action on rapidly dividing cells. Examples are:-

- a) Neurotoxicity - this occurs most commonly with vincristine.
- b) Cardiotoxicity - this is a severe side-effect of daunorubicin and doxorubicin.
- c) Pulmonary toxicity - this constitutes the therapy-limiting toxicity of bleomycin.
- d) Hepatotoxicity - this occurs with several agents.
- e) Renal toxicity - this is common with high doses of methotrexate.

Additive toxicity in combination therapy may be avoided by using drugs which have side effects on different types of tissues. Malignant cells are usually not as efficient at repairing the damage caused by the drugs as normal cells. This is the reason why a rest period is often included in anticancer protocols, so that the normal tissue can recover. The tumour has less potential to recover, therefore the effect is theoretically as shown in Figure I. (Note that the cell kill is graphed as a line with a negative slope, expressed in logarithmic terms). In man with severe malignant disease, the number of tumour cells in the body may be greater than 10^{12} . The number of tumour cells killed by one course of a drug is likely to be between 10^2 and 10^5 , therefore it may take many courses of treatment to eradicate all the tumour cells, if that is even possible (Schabel, 1975, DeVita et al., 1975).

Drug Resistance

Another problem of cancer chemotherapy is drug resistance. It is often the case that the first course of treatment with a given drug is successful, but that if the patient relapses, a second course of the same treatment is much less successful. There are several possible reasons for this, such as selective survival of more resistant cell lines, decreased permeability of the tumour cell to the drug, improvement in the ability of the tumour cell to repair itself, use of alternative biochemical pathways, an alteration in the specificity of

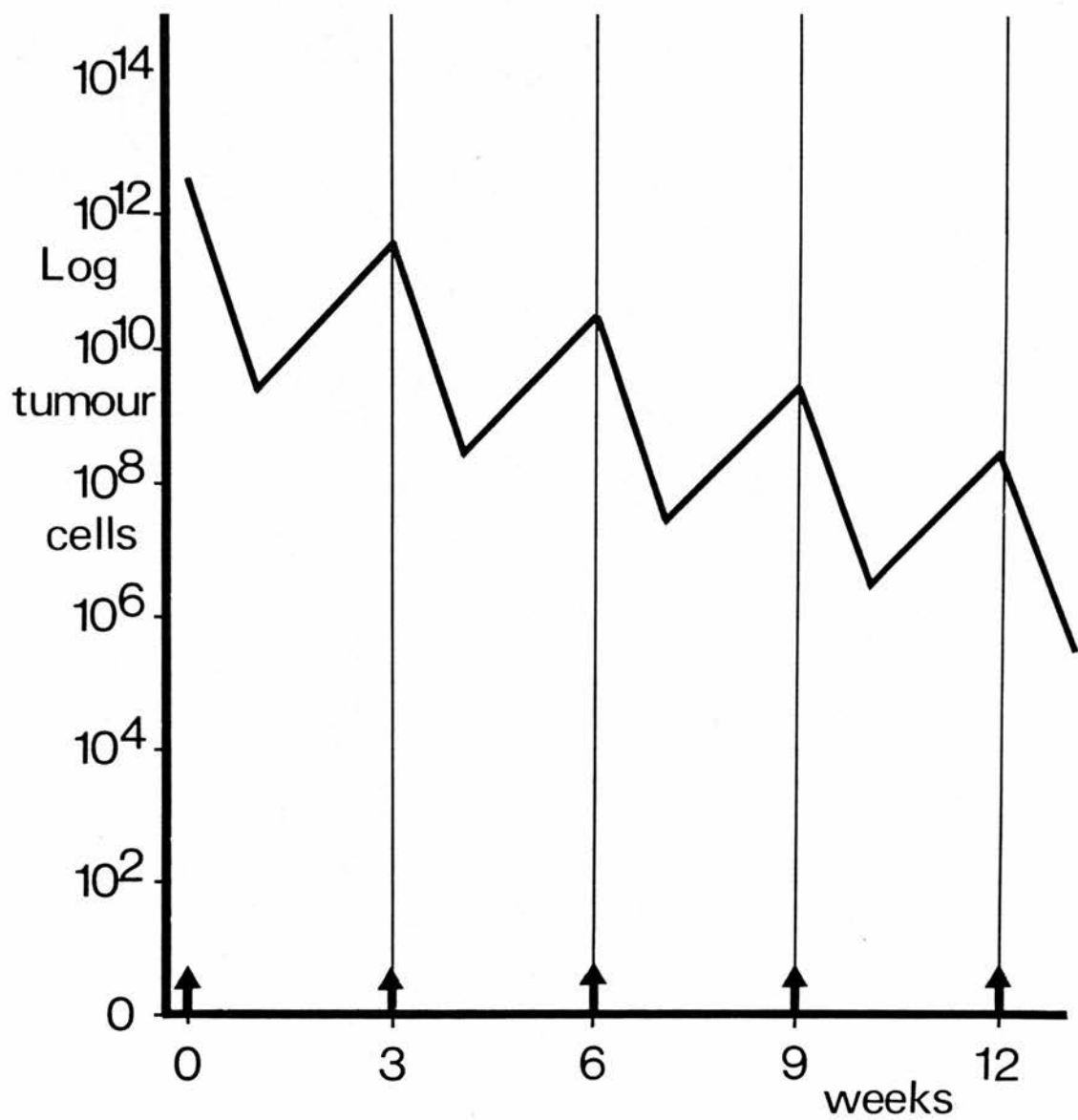


FIGURE I

Theoretical effects of five three-weekly doses of an anti-cancer agent on tumour cell numbers.

an inhibited enzyme or changes in the metabolism of the drugs. The end result is very similar to the development of resistant strains of bacteria. Combinations of drugs are often more successful than single agents as there is less chance of resistant strains of tumour cells developing.

Changes in the effects of the drugs may be seen when a different route of administration is employed, or when the dose or the schedule is altered or when a different formulation of the drug is given. The effects of the drug on the tumour depends on the degree of contact between the drug and the tumour cell. If the drug is carried in the blood-stream, it will have little

Note: Poor blood supply is not the reason why the testis should be a preferred tumour site, as drug damage is seen histologically. (Innes, personal communication).

Three methods of measuring a patient's response to cancer therapy are as follows.

- 1) No response (NR) partial remission (PR) and complete remission (CR). With this widely-used simple system, patients are considered to be in complete remission if all post-therapy staging studies, including bone marrow biopsy, are normal. A partial remission is defined as a 50% (or by some authors a 75%) decrease in the product of two diameters of all palpable

tumour. Most authors do not consider that a subjective improvement, or relief of pain, can be considered a response.

- 2) The method of Hewlett, Battle, Bishop, Fowler, Schwartz, Hagen and Louis (1964) for assessing response in the leukaemias. Patients are given a number on examination of bone marrow (M), haemogram (H), physical condition (P) and performance status (S) e.g. a complete remission would be M1, H1, P1, and S1. A good partial response would be M1 or 2, H1 or 2, P1 or 2 and S1 or 2.
- 3) The Karnofsky Categories (Karnofsky and Burchenal, 1949).

Category O - O	Disease progresses.
O - A	Subjective benefit without favourable objective changes.
O - B	Favourable objective changes without subjective benefit.
O - C	Favourable objective changes + subjective benefit (in measurable criteria) but of less than 1 month's duration.
Category I - A	Distinct subjective benefit with favourable objective changes in all measurable criteria for one month or more.

- Category I - B Objective regression of all palpable or measurable neoplastic disease (all lesions be reduced at least 50% in bulk) for 1 month or more in a relatively asymptomatic patient.
- I - C Complete relief of symptoms if any, and regression of all manifestations of disease for one year or more.
- Category II Interruption or slowing in the progression of the disease without definite evidence of subjective or objective improvement.

Veterinary Chemotherapy

Veterinary medicine has lagged far behind human medicine in the investigation into therapy of tumours in the domesticated species. The most important reasons are the high cost of treatment in relation to the economic value of a farm animal, an unwillingness on the part of a pet animal's owner to consider treatment which will probably result only in a temporary improvement in the animal's condition and the frequent development of side-effects caused by cytotoxic drugs. Nevertheless, in recent years the specialty of Veterinary Oncology has begun to develop especially in the United States of America. As domestic pets now tend to live

longer because of better control of infectious diseases, it seems likely that the treatment of cancer, particularly in the dog and cat, will become more sophisticated.

Most of the early attempts at chemotherapy of tumours were in dogs. In 1946, Innes, Parry and Berger treated a Golden Retriever with lymphosarcoma, using urethane. They kept it alive and reasonably well for 58 days, which is remarkable because they considered the dog to be near death when treatment was started. Bloom (1952) showed that corticosteroids could produce transitory regression of inoperable canine mast cell tumours.

In 1953, Jennings treated two dogs with urethane, one of which survived 20 days, and one with urethane plus nitrogen mustard, which was destroyed at the owner's request after 31 days. All three had lymphosarcoma.

McCoy, Allison, Crossley and Wannemacher (1956) used N (3- Oxapentamethylene) -N₁ N - diethylene-phosphoramidate (MEPA) in 25 cases of neoplasia in dogs, including seven cases of lymphosarcoma. All except one of these seven showed regression of the tumour, but severe drug toxicity was responsible for death in most of the cases.

Clover (1958) successfully treated a Scottish Terrier with lymphatic leukaemia using prednisolone, and Agresti (1959) reported clinical and haematological improvement in a dog with lymphatic leukaemia treated

with triethylenemelamine, prednisolone and adrenocorticotrophic hormone (ACTH).

Jacquier (1963) recorded his attempts to treat six dogs with various types of leukaemia with corticosteroids, cyclophosphamide and multivitamins, and McClelland (1963) described results with cyclophosphamide in four cases of canine lymphosarcoma. One of these achieved complete remission, but died, presumably from drug toxicity.

Lymphosarcoma appears to be the tumour most commonly treated by chemotherapy, and since 1963, there have been many references in the literature. As in humans, combination chemotherapy has been found to be more successful than single agent therapy in canine lymphosarcoma. A variety of drugs have been used by various workers and no one regime has emerged as superior to the others, though corticosteroids, cyclophosphamide and vincristine have been favoured in more than one trial. See Table 2.

Cohen, Schmidt, Lucas and Palmer (1975) used these three drugs to treat beagles with transplanted canine lymphoma, and this produced long term remissions. Chemotherapy of lymphosarcoma in cats has not often been attempted because of the probability that the disease is caused by Feline Leukaemia Virus infection. Affected cats are therefore a danger to others and are usually destroyed. ^{and Norsworthy} Freitag/(1976) treated two Siamese cats successfully with vincristine and prednisone. These were considered suitable cases for therapy because they

TABLE 2.

VETERINARY CHEMOTHERAPY

<u>AUTHORS</u>	<u>No. of cases treated</u>		<u>Drugs Used</u>	<u>(MST or MRT)</u>
	<u>Dogs</u>	<u>Cats</u>		
Moldovanu, Friedman and Miller (1966)	14	0	C	MST-110 days
Old, Boyse, Campbell, Brodey, Fidler and Teller (1967)	3	0	L-A	MST-33 days + one still alive at 50 days
Brick, Roenigk and Wilson (1968)	4	1	C	MST- 4.5months
	6	2	P	2.5months
	9	2	Chl.	3.5months
	7	1	P+Chl.	2.3months
Squire and Bush (1973)	100	15	P	MST-Dogs -156
			C	days
			V	Cats-not stated
			O	but all dead
			6-M Ara-C	within 3 months
Squire, Bush, Melby, Neely and Yarborough (1973)	49	0	P	MRT- 53 days
	34	0	P	62 "
	19	0	+C	
	25	0	COP	184 days
		0	COP 6-M	136 days
Madewell (1975)	20	0	Induction COP	MST-211.5 days (1 case ex- cluded)
			Maintenance C+M+6M	MRT of respond- ers 104.8 days
Theilen, Worley and Benjamini (1977)	47	0	Single drugs:-	
			P	MST < 60 days
			C	
			O	
			Combinations	
			of:-	
			P	
			C	
			O	MST - 138 days
			Ara-C	
MacEwen <u>et al.</u> , (1977)	38	0	A	
			M	
			L-A	
			Combinations	
			of:-	
			O	
			C	
			Ara-C	MST - 132 days
			M	
			L-A	

<u>AUTHORS</u>	<u>No. of cases treated</u>		<u>Drugs Used</u>	<u>(MST or MRT)</u>
	<u>Dogs</u>	<u>Cats</u>		
Owen and Bostock (1978)	48	0	Combinations of:- O Ara-C C P	MST - 6months 36 responders- MRT - 15 weeks

Abbreviations - P - Prednisolone, C- Cyclophosphamide, V-Vinblastine,
O- Vincristine, L-A - L-asparaginase, M- Methotrexate, 6-M - 6-mer-
captopurine, Ara-C - cytarabine, Chl- Chlorambucil, A- Adriamycin.

MST - Mean survival time

MRT - Mean remission time.

were negative to the commonly used serological test for FeLV. He concedes, however, that they may have had FeLV in their tissues even though it was not detected.

As the mean survival times in Table 2 indicate, none of the authors could report outstanding success. Survival times of untreated cases vary greatly, partly because of delays in diagnosis or delay by the owner in taking the animal for examination. Madewell (1975) has summarised survival times of untreated cases in dogs as reported in the literature. The mean survival times quoted vary from as short as 9.8 days to 99.5 days, therefore it would seem that in most chemotherapy trials there is a definite prolongation of life.

Cancer chemotherapy in domesticated species other than the dog and cat has only been attempted very rarely. In the larger species, the size of the animal means using large volumes of drugs which makes the cost prohibitive. Ward and Whitlock (1967) tried chemotherapy in a case of equine leukaemia with amethopterin.

Hereditary Lymphosarcoma - Its Value as a Model of Human Disease

New anticancer drugs are continually being produced by research chemists, but because of the probability of severe side-effects, the use of these agents must be restricted until they have been extensively tested in vitro and in laboratory animals.

There are two major aims in drug tests i) to predict toxicity and ii) to predict efficacy.

(1) The animals most commonly used are rodents such as rats and mice, rabbits, guinea-pigs, and the dog. Monkeys are also frequently used. There are three principle reasons for the initial toxicity trials. Firstly, compounds whose toxicity is likely to be unacceptable, i.e. those which produce lifethreatening toxic effects in all species, can be eliminated. Secondly, essential pharmacological information must be gained so that a trial dose may be calculated. Thirdly, details of the toxic effects in different species, are ascertained, how variable they are and whether or not they can be reversed. In this way, toxic effects in man can supposedly be predicted, though there have been cases of drugs which produced unexpected adverse reactions in man after being tested in laboratory animals. Reasons for this may be,

- a) that the experiments performed were inadequate,
- b) that there are marked species differences in response to the drug,
- c) that human cancer patients are often in poor general condition, possibly/impairment of function of organs

such as liver and kidneys, and may have been given previous toxic therapy, whereas only healthy animals are used for toxicity tests.

In many laboratories, the procedure of finding the dose of a drug which is lethal to 50% of the animals tested is carried out. This is known as the LD 50 for that drug, in that species of animal and it is used as a basis for calculating an initial clinical dose. In other centres, four dose levels are determined. These are a) the highest nontoxic dose, b) the lowest dose which produces pathological effects of any kind, c) the dose which produces toxic signs and when doubled causes death, and d) the lowest dose which causes drug-induced death in any animal during treatment (Creaven and Mihich, 1977).

(2) The procedures for testing the efficacy of an anticancer drug are not quite so well defined. As mentioned previously, the effects of a drug are modified greatly depending on the mode of administration, the formulation and alterations in metabolism, and its effects on the tumour depend on whether or not the drug has adequate contact with the target cell. The host immune response, the functions of lymphocytes and macrophages against the tumour must not be forgotten - these functions may be altered by the drugs, as most are immunosuppressive. When these problems are considered, it would seem that in

vitro tests, such as the effects of drugs on tumour cell lines in tissue culture, are fairly limited in their usefulness for predicting the effectiveness of drugs in cancer therapy, though they may provide important data about the actions of various drugs in the different stages of the cell cycle.

The obvious requirement, therefore, is a suitable animal model of the tumour against which the drug is intended to be used. Scientists have been searching for many years for animal models of human tumours, and relatively little progress has been made in the treatment of human cancers which do not have common animal equivalents. The neoplasms of the laboratory rodents, to which I have already referred, have been very useful in the advancement of knowledge about leukaemias and lymphomas and their treatment, but they are too different from man to provide anything other than basic information. Many scientists, doctors and veterinary surgeons now believe that tumours in the larger domesticated species should be studied, not only because they are interesting curiosities, but because they could be important as models of human cancer. Professor Cotchin in his presidential address to the Royal Society of Medicine in 1976, gave some of the reasons why he thought that tumours in animals are still not being investigated from the comparative aspect with the attention that they deserve. One of these was that there is a popular belief that successful laboratory work on cancer demands the use of

highly inbred strains of experimental animals. He pointed out however that it is questionable how immediately relevant work on experimentally induced or even spontaneous tumours occurring in these animals is to the spontaneous cancers occurring in largely out-bred human populations. Another reason was that the practical problems of studying tumours in domesticated animals are difficult to overcome: the animals are much more expensive to keep because they are larger than mice, and trained investigators are few. However against the problem of size and expense must be weighed the advantages - more tissue, blood and other material is provided for analysis, the tumour may be accessible at all stages of its development, and the animal is much more suitable for therapeutic studies because of its larger life-span.

The dog is considered by many to be the best species for use as a model, e.g. Engstrom, Shumway, Jones, Bertino (1965), Owen, Bostock, Beiton, Onions, Holmes, Yoxall and Gorman (1975) but the major problem is that, as mentioned previously, most canine tumours are sporadic, of unknown aetiology and tend to occur in old age. Cases for study are usually obtained by cooperation with veterinary surgeons in general practice who may refer them to a centre with the expertise and facilities for therapy. However owners are often unwilling to have their animals used for experimental work, therefore the number of cases is bound to be limited. The work which is being done on transmitting canine lymphoma, as already

discussed, may solve this particular problem. However as with other induced tumours, an artificial situation is created which may not be identical with the naturally-occurring disease.

Neoplasms of many kinds, and haematopoietic ones in particular, are very common in the cat, but its usefulness as a model may be limited because of the tendency of this species to have unusual reactions to many drugs. Although cases of feline lymphosarcoma are readily obtained in the laboratory, the presence of Feline Leukaemia Virus means that the pathogenesis of the disease may well be different from that in species such as man in which a virus has not so far been implicated.

The other main problem which will always exist, in Great Britain at any rate, is that the use of cats and dogs for experimental purposes, whatever the benefits to be gained from it, is a very emotive subject.

The pig is a domestic species which resembles man in several ways. It is comparable in body weight, monogastric, omnivorous, and relatively hairless. On the other hand the gestation time is quite short (114 days), litter size is large with a survival mean of about 10, and with good management two litters can be produced in a year. Hereditary lymphosarcoma is a naturally occurring pathologically homogeneous condition which resembles closely poorly-differentiated lymphocytic lymphoma of man. Being

associated with an autosomal recessive gene it can be produced simply by the mating together of two known carrier animals, when, on average, 25% of the progeny are affected.

Because of these facts, it was considered that affected pigs might be suitable for use as a model for testing the antitumour effects of new drugs before they were introduced for clinical trials in man. They could also be used to increase the present knowledge of the pharmacokinetics and scheduling of drugs which are already in use in man. However it was necessary first of all to show that the pig, and in particular the lymphosarcomatous pig would respond in the same way as man to the different types of cytotoxic drugs already in common use.

Reichel (1962) appears to be the only author to have reported the effects of chemotherapy on lymphosarcoma of the pig. Published work on the effects of cytotoxic drugs in the normal pig appears to be scarce. The use of corticosteroids, adriamycin and cyclophosphamide has been reported (See Chapter I, II and III). The purpose of the project documented by this thesis was to investigate:

- 1) The clinical, haematological, biochemical and pathological effects of some cancer chemotherapeutic agents in the normal pigs. It was not to determine an LD₅₀, which would have required large numbers of animals and much more extensive studies, but merely to establish, if possible, a dose which would be effective but relatively non-toxic.

- 2) To discover whether or not these agents would produce remission in the lymphosarcomatous pigs.
- 3) To determine how closely the effects of various agents on pigs were comparable to their effects on man.
- 4) To demonstrate whether or not hereditary porcine lymphosarcoma is a suitable model for research into the effects of new antitumour agents intended for use in the therapy of lymphoma in man and companion animals.

Choice of Drugs

A wide variety of drugs could have been used in this study, and the ones which are recognised to be most useful in the treatment of cancer in man are shown in Table 3. Those marked with an asterisk (*) were chosen because they have been shown to be particularly effective in cases of human lymphoma and leukaemia, and there is a great deal of information about them in the literature (Cline and Haskell, 1975) .

Cytarabine was chosen from the antimetabolite group because lymphoid tissue from the lymphosarcomatous pigs had been shown to contain high levels of deoxycytidine kinase (Baxter, personal communication - See Chapter IV).

TABLE 3
Anti-tumour chemotherapeutic agents

Cortico steroids	Alkylating agents	Antimetabolites	Antibiotics	Other
* prednisolone	nitrogen mustard	methotrexate	actinomycin-D	vincristine
prednisone	*cyclophosphamide	*cytarabine	*doxorubicin (adriamycin)	vinblastine
betamethasone	melfhalan	5-fluorouracil		procarbazine
dexamethasone	busulphan	6-mercaptopurine	daunorubicin	nitrosoureas
	ifosfamide	thioguanine	bleomycin	L-asparaginase
	chlorambucil		mithramycin	razoxane
			mitomycin-C	

*Those marked with asterisks were used in the trials documented by this thesis.

MATERIALS AND METHODS

The materials and methods used were basically the same throughout the whole series of experiments, though not all the measurements and techniques were necessarily carried out in every experiment. Techniques which were only used for experiments using one drug are described in detail in that chapter.

For each drug used as a single agent, there was a trial using normal pigs and a therapy trial using lymphosarcomatous pigs.

Trials in normal pigsAnimals

The first trial used eight pigs divided into four groups of two - one pair for a high dose level, one pair for a medium dose level, one pair for a low dose level and two saline - treated controls. After the results of this first experiment were analysed, it was considered that two control pigs were not sufficient, and in all the subsequent trials using normal pigs, ten were used, four of which were saline-treated controls. Initially, it was thought desirable that all the pigs used in these trials should be from the same litter, so as to eliminate as much as possible, genetic variation in response to the drugs. However, this created a problem because in a large litter there was, usually, a "runt" pig, i.e. one which was smaller and less vigorous than the others, for

a variety of reasons. The results from a "runt" animal tended to differ from the results obtained from the other pigs, and so it was decided that only the healthiest and fastest-growing pigs from two closely related litters, born at approximately the same time, should be used if possible. All normal pigs were from the lymphosarcoma strain, but were from non-carrier dams and, if possible, sired by non-carrier boars.

All the pigs used were bred in a piggery which was modernised for the purpose, using breeding stock transferred from the older minimal disease piggery referred to in the Introduction. The disease precautions practised in both piggeries were the same. Only authorised personnel wearing protective clothing were allowed access.

The normal experimental pigs were weaned at 6 weeks of age, provided they were well grown and consuming an adequate quantity of creep feed. Before the experiments started, they were introduced to a diet of pig nuts (Breeder's Cubes, SAI Ltd., Edinburgh) with the following analysis:-

Vit. A 11,000 iu/kg

Vit.D₃ 2,000 iu/kg

Vit. E 10 iu/kg

Salt - 0.64%

Lysine - 0.55%

Methionine and

cysteine 0.5%

Oil	-	3.26%
Protein	-	15.5%
Fibre	-	5.82%
Calcium	-	0.9%
Phosphorus	-	0.61%

All pigs used in one experiment were housed together in the same pen. Free access to water was provided by an automatic water bowl.

Male pigs were castrated before weaning, usually at about 3 weeks of age.

Delayed hypersensitivity skin tests

Since Aisenberg's studies (1962) in Hodgkin's disease patients, dinitrochlorobenzene, and the related compound dinitrofluorobenzene (DNFB), have been used as skin contact antigens for the study of cell-mediated immunity in lymphoid malignant disease.

At about 7 or 8 weeks of age, all the young pigs were sensitised to DNFB and tested in an identical manner 2 weeks later.

The method was as follows. The pig was restrained and a patch of hair was removed from the skin of the back using surgical scissors (the most usual areas were in the mid-line between the tops of the shoulder blades, or the sacral region.) The skin was thoroughly cleaned with a cotton-wool swab soaked in methylated spirit, to remove dirt, skin scales and grease, and the area was

dried with another piece of cotton wool. A small round area of 14mm diameter was defined using a short piece of glass tube, and 5 μ l of 10% DNFB in equal volumes of dimethylsulphoxide (DMSO) and acetone was dropped inside the tube using an automatic micropipette with disposable tips. The DNFB solution was spread using the flattened end of a glass rod within the area, and allowed to dry before releasing the pig. Rubber gloves were worn by the operators to prevent self-sensitisation.

Observations on the state of the skin, to which the DNFB had been applied, were made at 24 hours and 48 hours and were recorded using the following arbitrary scale:-

0	1	2	3	4
no change	slight erythema	erythema+ slight thickening	marked ery- thema with vesiculation or small ul- cerations	severe ulcer- ation and bleeding

Skin tests were repeated at intervals during the experiments, always at the end of treatment and after recovery from the effects of the drug. The response to these tests gave a measure of cell-mediated immunity.

Body weight

At about the same time as skin sensitisation, weekly weighing was started, the weight being recorded to the nearest 0.5kg using a pig weighing-crate (Kwikway Products & Co. Ltd., Birmingham). This was continued to the end of the experiment.

Collection of blood samples

Shortly after this, blood-sampling was started. Before

treatment in all experiments, this was carried out weekly, at the same time of day, usually at 9.30 a.m. During treatment samples were taken weekly, or more frequently, depending on the drug.

The pigs were restrained by blood sampling either in dorsal recumbency, or held on their feet by means of a rope noose round the upper jaw, depending on their size.

Up to approximately 40 kilograms body weight, the young pigs were placed in dorsal recumbency on a V-shaped trestle of wooden boards held by a metal framework on metal legs 3 feet high. With one hand pressing the head down and the neck stretched, a blood sample was obtained from the anterior vena cava using a 10ml disposable syringe and a 19 or 20 gauge $1\frac{1}{2}$ or 2 inch needle, or occasionally using "Vacutainers" (Becton-Dickinson). The skin was cleaned with cotton wool soaked in a 1 in 1000 dilution of "Hibitane" in surgical spirit. The point of entry of the needle was in the right supraclavicular fossa about $\frac{1}{2}$ to 1 inch cranial to the manubrium of the sternum, the needle being directed caudally, dorsally and towards the mid-line. Part of the sample was put into a plastic EDTA sample bottle (Searle) for haematological examination, and the remainder put into a dry 10ml glass test-tube with a rubber stopper for serum. Haemorrhage after sampling was usually easily controlled by pressure using a pad of dry cotton wool, but if a haematoma developed at the site, the next sample was

taken from the left side in the same manner. While drawing the sample into the syringe, excessive suction had to be avoided so that the red cell haemolysis did not occur. Over 40kg body weight the pigs were tipped on to their backs and restrained in a modified ground level V-shaped cradle (McTaggart, 1977).

Most of the pigs were not allowed to reach body weights greater than 60kg but occasionally it was necessary to restrain a pig by means of the rope noose, tied to a suitable firm object or in a handling crate. In these cases, blood samples were taken using EDTA and plain vacutainers from an ear vein, raised by means of a rubber band round the base of the ear. Even less frequently a small blood sample was obtained by puncturing a vein with a 16 gauge needle near the edge of the ear after clipping the hair, cleaning the skin with cotton wool soaked in surgical spirit, ^{and} applying petroleum jelly to the skin surface. Blood was then allowed to flow into the tube. The methods used in handling of blood samples in the laboratory will be dealt with later.

Clinical examinations

In addition weekly clinical examinations were carried out before the start of drug treatment. Rectal temperatures were taken using a lubricated mercury clinical thermometer or an Ivac 821 (Loxley-Luxan) electronic thermometer in heavier pigs which were more difficult to restrain. This was done at the same time of day each

time, but there was still a variation in temperature readings obtained. It was possible however to ascertain an approximate "normal" range for each pig, so that the ~~sig~~ relevance of ^{any} marked alteration in rectal temperature during drug treatment was appreciated. Respiratory and pulse rates were not accurately measured as they were considered to be too variable, due to excitement, to be of much relevance. However, each pig's thorax was auscultated to detect any abnormalities in lung sounds or cardiac sounds, before treatment was started. The body condition of each pig as well as the state of its appetite was noted, also the appearance of its faeces and urine. Any injuries or other abnormalities were noted and treated if possible before the start of the experiment. Occasionally, a pig was found to have developed a condition which made it unsuitable for inclusion in the experiment. It was rejected and another pig substituted.

During drug treatment, the pigs were observed every day for signs of ill health, particularly loss of appetite and soft faeces, and they had a similar complete clinical examination to that carried out before treatment once every week.

All the trials using normal pigs followed the same basic pattern with half of the pigs being destroyed and autopsied at the end of the prescribed period of treatment, which varied according to the drug, i.e. one high

dose pig, one medium dose pig, one low dose pig and one (of two) or two (of four) controls were killed. The remaining pigs were left untreated until it was felt that all the effects of the drug had disappeared, when they too were destroyed and autopsied.

Therapy Trials

Animals

Each litter resulting from the mating of a carrier boar and a carrier sow was checked for enlargement of the superficial inguinal and precrural lymph nodes at 6 or 7 weeks of age. Any animals showing suspicious signs were blood sampled at this stage for haematology. Sometimes, there was no evidence of disease at this stage. The litters were checked at 8 or 9 weeks by which time most cases were showing signs. One more check at 10 or 11 weeks was usually carried out to be sure that no cases had been missed. In general, diagnosis presented few difficulties except where there had been a problem with intercurrent disease in the litter.

Once cases were definitely identified, they were separated from their normal littermates. Males transferred from the older piggery were castrated. Those bred in our own piggery were left entire.

On 16th November, 1977 the lymphosarcoma animal house was opened, and cases were moved to this building. Prior to this date, lymphosarcomatous pigs were all kept together in one pen in the breeding piggery. Introducing animals of different sizes never caused problems as these

pigs did not fight as normal pigs tend to do when mixed with other strange pigs. However, there were disadvantages. Individual feed intakes could not be assessed or controlled, and it was difficult to make observations on the state of faeces and urine for individual pigs. There were no facilities for surgery, other than very minor operations. No heat source could be provided in the pen, and the pigman had no time to nurse sick animals.

Accommodation for pigs in the new house comprised five loose boxes, which were each subdivided into three individual pens with walls 5 feet high between them, and solid metal gates with sliding diagonal bolts. The floors of the pens had raised bedding areas at the back and dunging areas at the front, each of the three pens draining to the centre of the entrance area of each loose box. The floors were insulated but straw was also given and heat lamps provided for use when pigs were sick, or after surgery when recovering from anaesthesia. Nipple drinkers were situated on the walls near the doors to encourage defaecation and urination in the correct area by keeping the floor wet. No built-in mangers were provided. Metal moveable mangers were found to be unsatisfactory and so the pigs' food was placed on the floor in the bedding area.

Handling was carried out in the central passageway between the loose boxes. Trestles used were similar to those described for the trials using normal pigs, but

no crate was available for handling larger animals. Two metal rings were inserted in the wall of the passageway and these were used to tie up larger pigs using a rope noose. Problems were encountered when the canine teeth were not large enough to keep the noose from sliding off and to overcome this, a harness was designed. This was made of heavy nylon strapping and was fully adjustable. It consisted of a strap around the body of the pig behind the fore-legs, attached to a strap over the shoulders, neck and between the ears with a ring on the end through which the rope noose was passed before tying up.

The pig food used was similar to that used in the breeding piggery, but when pigs' appetites were poor, a variety of other foods were offered including milk and fruit and vegetables. Careful nursing was found to be essential and undoubtedly saved pigs which would otherwise have died.

Disease staging

When cases were moved to the animal house, they were kept in a separate "quarantine" loose box as far from the inmates as possible for 3 weeks until their condition could be properly assessed. Once the pigs had become accustomed to the new accommodation, they were prepared for laparotomy. This procedure confirmed the diagnosis. Disease staging in man is an essential procedure before commencing therapy, and is usually defined according to the Ann Arbor Staging Classification, (See Introduction, Table 1).

In man, staging laparotomy is usually carried out after biopsies of bone marrow and liver, radiography of chest and abdomen. Radiography was not possible with the pigs as X-ray apparatus could not be brought into the piggery and the pigs could not be moved. However, although it was important to ascertain the degree of involvement of various organs in individual pigs before therapy started, it was known that they were all likely to be in Stage IV by the time of diagnosis, and so the omission of these procedures was considered unimportant. The main reasons for laparotomy were to obtain mesenteric lymph node biopsy material, to demonstrate by photography the effects of the drugs, if any, on mesenteric nodes and spleen by repeating the procedure immediately after therapy was stopped and to simulate the procedures used in human patients.

Laparotomy Technique

Preparation

The pig was starved the day before surgery. On the morning of the operation, the animal was given azaperone ('Suicalm', Janssen) intramuscularly at a dose rate of 4mg/kg body weight. Twenty minutes was allowed to elapse so that this tranquilliser would produce its maximum effect. The pig was then gently restrained in ventral recumbency on the high bleeding trestle, one ear

was cleaned with methylated spirit-soaked cotton wool and a 2.5% solution of thiopentone sodium ('Intraval', May and Baker) was injected intravenously to effect using an approximate dose of 20mg/kg, through a 23 gauge 'butterfly' Miniven set' (Portex Ltd., Hythe). When sufficient jaw relaxation had been achieved and the 'gag' reflex had been abolished, the mouth was held open by an assistant, using two pieces of bandage, one piece round the lower jaw, under the tongue and the other round the nose and upper jaw. A rubber or plastic cuffed endotracheal tube (various makes, 6.0 to 11.5 millimetre, depending on the size of the pig) was inserted, with the aid of a laryngoscope. Once it was satisfactorily in place, which was confirmed by feeling air rushing down the tube as the animal breathed, the cuff was inflated and the tube tied in place with the piece of bandage round the upper jaw.

The pig was then moved to the operating theatre and placed on the table in dorsal recumbency but tilted slightly to the left. It was held in position with sand bags. The endotracheal tube was attached to a anaesthetic machine with vaporiser. A halothane ('Fluothane', I.C.I. Ltd.) and oxygen mixture was used to maintain and deepen anaesthesia slightly so that surgery could begin. Nevertheless, the plane of anaesthesia was kept as light as possible, since respiration was often poor, and the heart rate was monitored

frequently in case of cardiac arrest. During the first series of operations, one pig died due to shock and failure of venous return. Pressure on the diaphragm caused by the huge volume of abdominal organs and tumour tissue, in addition to the poor general condition of the animal, was thought to be responsible, and in subsequent operations the table was tilted about 20 degrees so that the pig's head was higher than its tail. This was very successful. Respiration improved and there were no more anaesthetic deaths. The air temperature in the operating theatre was kept as high as possible, and hot water bottles were arranged round the pigs during and after the operation in case of hypothermia. An area of skin around the intended site of the incision was shaved using shaving foam and a safety razor and the skin was disinfected with a 1 in 10 solution of "Hibitane" 5% concentrate in 70% alcohol.

Operating Procedure

The surgeons wore sterile gowns and disposable surgical gloves. Four sterile green calico drapes were held in place with towel clips around the site of the incision, which was longitudinal, parallel to the midline, from about 5 centimetres caudal to the costal arch to a similar distance cranial to the fold of the flank. This site was chosen in males to avoid the prepuce. When operating on the first female, a mid-line incision was tried, but the approach to the small intestine was more

difficult and the internal sutures broke down resulting in a large hernia, and so in all subsequent operations the paramedian incision was used. The incision was made with a scalpel and the aponeurotic sheets of the external oblique, internal oblique and transverse abdominis muscles were cut through one by one with straight scissors. Bleeding points were swabbed and then crushed with artery forceps, and, if necessary, tied off with catgut. The peritoneum was exposed, lifted with rat-tooth forceps and nicked with scissors. Peritoneal forceps were attached on each side, and then the peritoneum was also cut along the incision line with straight scissors, taking care not to cut any organs underneath. Tissue forceps were then applied to the peritoneal and muscle layers on each side, for better grip. The loops of the small intestine were exteriorised. If possible, the terminal part of the ileum with the characteristic hook-shaped lymph node, were brought to the incision, and laid on the drapes for photography. If, however, the incision was fairly far forward, this part was difficult to find and, to save excessive handling of the intestine, a more accessible part was photographed. A piece of lymph node was then removed, for histological examination. The peritoneum covering it was slit and a piece was removed by blunt dissection, controlling any haemorrhage, which was usually slight, by pressure, and then the peritoneum was closed again by a continuous suture with catgut (straight needle attached). The loop of intestine was then returned to the abdominal

cavity. The next stage was to exteriorise as much of the spleen as possible without pulling too much on it, as in most cases it was very enlarged and rather friable. The spleen was also photographed. For all photographs, a metal rule marked in centimetres and millimetres was placed beside the lymph nodes and spleen. By this method, it was hoped to be able to demonstrate the effect of the drugs on the nodes and spleen. However, in many photographs, the metal rule reflected the flash light and the numbers were unreadable.

Finally the abdomen was closed. The peritoneum and muscle layers were closed with a continuous suture of catgut using a curved round-bodied needle. The skin incision was closed with interrupted mattress sutures of nylon with or without a cutting needle attached using a pair of Gillie's needle holding forceps.

An injection of long-acting benzathine penicillin ('Penidural', Wyeth) was given intramuscularly after the operation. The anaesthetic machine was switched off and the pig disconnected from it. The animal was then returned to its pen with the heat lamp switched on, to recover from the anaesthetic. By this time most animals were beginning to show signs of recovery, therefore the cuff was deflated and the endotracheal tube was removed. The pig was made to lie on its sternum using sand bags to support it. Cases which had undergone

surgery were usually drinking by the same evening, and eating a little by the next day. Healing of the wound was usually uneventful but one or two post-operative complications arose, which will be described in the appropriate chapters later on.

Pre-treatment routine

Clinical observations were recorded daily and detailed clinical examinations were carried out weekly. These examinations included taking the rectal temperature, auscultation of heart and lungs and careful examination of eyes, mouth, skin and feet. Measurement of the lengths and breadths of the superficial inguinal and precrural lymph nodes were carried out, using a pair of tuberculin-testing calipers, without lifting the nodes into a fold of skin. When pigs were in remission, it was frequently impossible to measure the nodes. Even when they were quite large, it was often difficult to measure the precrurals with accuracy, but since the procedure was carried out in exactly the same way each time it is to be hoped that the error was fairly constant. The circumference of the abdomen was measured at the level of the umbilicus using a measuring tape at the end of expiration. Initially the reason for taking this measurement was that with certain drugs the pigs' abdomens seemed to become more pendulous than they were before the start of therapy. However, with these measurements, which were taken with the pig in dorsal recumbency, it was possible to estimate whether this apparent enlargement

was real or not. In fact they proved to be useful as an indication of the state of the tumour. (See Results).

Before therapy was started, the pigs were photographed standing in front of a black board, marked off in squares with white tape, or in front of the grey-painted door of the animal house, which was marked off in identical squares with black tape. They were also photographed in dorsal recumbency with the hind legs frogged and held well apart to display the inguinal lymph nodes.

Skin sensitisation and testing were performed and blood sampling and weighing were carried out, as described for the trials in normal pigs.

Procedure during therapy trials

Four pigs were used in each experiment, except for the trials using corticosteroids in which an extra two pigs were used, for reasons which will be explained later. The dose rates used, methods of dosing and duration of experiments were different for each trial and will be described in detail in the appropriate chapter. Weekly weighing, bleeding and detailed clinical examinations were continued during therapy. In some experiments, it was necessary to blood sample more frequently, usually to monitor white blood cell counts. Clinical observations were made daily on fitness, appetite, whether faeces were soft, hard or

diarrhoeic and any other details that were noticed. The most frequent problem encountered was diarrhoea. This was often severe enough to cause rapid loss of condition and dehydration especially in small pigs, and even death on one or two occasions, therefore treatment was usually given without delay. If the pigs were afebrile and small enough to be easily dosed by mouth, the usual mixture administered was 'Streptaquaine', (Elanco Products Ltd.) 15 to 20ml/day. This contains 1.76g dihydrostreptomycin sulphate, 1.76g sulphadimidine and 21.1g light kaolin per 100ml. The dosing apparatus was a 20ml. syringe attached to a piece of polythene tubing about 25cm long, with rubber tubing inside it and turned out over the sharp end to protect the pig's pharynx.

In stubborn cases, or those in which the diarrhoea recurred, sulphadiazine powder with trimethoprim (Tribrissen, Burroughs Wellcome & Co. Dispersible powder), or neomycin sulphate powder ('Neobiotic' Upjohn Ltd.) or framycetin sulphate (Framomycin Feed Additive C-Vet. Ltd.) were given mixed with the feed. The dose rates were 0.25g/kg, 0.2g/kg and 10mg/kg respectively. 'Strep-Sulpha³taquaine' was given once or twice daily for 3 days and in-feed powder for 5 days or longer. Framycetin sulphate was used mainly when the pigs were in the breeding piggery and was administered daily at prophylactic dose level (5mg/kg) for the duration of the

experiment, because it was considered that challenge from infection was much greater in that situation. If the pigs were febrile and off food, antibiotics were given by intramuscular injection. A variety of preparations were used. These were:-

- (1) Ampicillin (150mg/ml) ('Penbritin', injectable suspension, Beecham Animal Health) dose rate 5mg/kg bodyweight, for 3 days.
- (2) Oxytetracycline, 50mg/ml ('Terramycin Q-50', injectable solution, Pfizer Ltd.). Dose rate 2 to 9mg/kg for 3 days.
- (3) Chloramphenicol (150mg/ml) ('Ertilen', Ciba-Geigy (U.K.) Ltd.). Dose rate 30mg/kg body weight for 5 days. This was only used in very severe cases.

Occasionally, faeces samples were submitted for bacteriological examination but it was already known that the likeliest infectious causes of intestinal problems were pathogenic strains of Escherichia coli, and Campylobacter sputorum salis var. mucosalis, the latter also allowing opportunist pathogens including spirochaetes and the protozoan parasite Balantidium coli, to gain a hold. Campylobacter sputorum salis has been implicated in a variety of conditions of the intestine of the pig (Rowland and Lawson, 1975).

If cases were severely dehydrated they were given fluid replacement therapy intravenously and subcutaneously with sterile glucose saline solution.

Other disease problems occurred much less commonly and will be described, along with treatment given, elsewhere.

At the end of therapy, skin tests and photographs were repeated. Half of the pigs were then destroyed, as in the normal pig trials, by an intravenous injection, into an ear vein, with 20% pentobarbitone sodium ('Expiral', Ceva Ltd.). These pigs were autopsied. If the remaining pigs had had laparotomies before therapy, this was repeated. Thus it was possible to confirm whether all the pigs in the experiment responded in the same way.

The two remaining animals were left untreated until all the effects of the drugs had apparently disappeared. By this time, the tumour had in most cases returned to the same stage of development, as before therapy started.

Methods of handling blood samples in the laboratory

Every week, 10ml. of blood was taken from each animal, 2ml for haematology and 8ml for serum, as previously explained. The haematology sample bottles were agitated gently by hand for about 1 minute after taking the sample, to ensure that the anticoagulant was mixed well with the blood. In the laboratory, the sample bottles were placed on a rotating apparatus to mix them thoroughly before processing. The blood for serum was left to clot in the test-tubes on the bench at room temperature until the clot was well-formed and sufficiently retracted not to adhere to the glass when the tube was

inverted. The sample was centrifuged hard for 30 minutes and the serum decanted into another tube. Residual red cells were removed by another half an hour's centrifugation and the serum was decanted again into plastic conical test-tubes.

Haematological techniques

White Blood Cell (WBC) Counts - (WBC) -

These were carried out using a Coulter electronic counter, (Coulter Electronics Ltd., Harpenden) Model ZF, using threshold setting 8 and sensitivity settings 0.707 and 16. Samples were diluted using the Coulter Dual Diluter III, and 6 drops of 0.3% potassium cyanide ("Zapoglobin") were added to the 20ml of diluted blood, to haemolyse the red blood cells. A mean of two successive counts at opposite polarities were taken and the figure obtained multiplied by 10^9 gave the number of WBCs per litre.

Differential Counts

A drop of well-mixed blood was placed at one end of a clean glass microscope slide, using a platinum loop. Another slide, with a corner cut off, was used as a spreader. A blood smear was prepared by allowing the drop of blood to run along the edge of the spreader, and then moving the edge of the spreader along the slide at an angle of about 45 degrees.

Blood smears were stained, using concentrated Leishman's stain for $2\frac{1}{2}$ minutes and then diluting it with

double the volume of buffered distilled water, pH 6.8, leaving this on the slide after mixing for 15 minutes. Slides were then washed with a stream of distilled water and left to dry at room temperature. Differential counts were based on 200 cells counted using a 54 times magnification oil immersion objective on a light field microscope (Leitz) with a blue filter. In blood smears from lymphosarcoma cases it was not easy to differentiate between the various types of mononuclear cells, therefore "total mononuclear cells" were counted.

Red Blood Cell Counts (RBC)

These were also carried out on the Coulter electronic counter, with the same settings for threshold and sensitivity as for WBC counts. A mean of two counts was taken and multiplied by 10^{12} to give the count per litre.

Packed Cell Volume (PCV)

PCV was measured using microhaematocrit tubes, centrifuge and reader (Gelman Hawksley, London), the samples being centrifuged for 5 minutes.

Haemoglobin (Hb)

Hb was estimated by the cyanmethaemoglobin method, using a Coulter Haemoglobinometer.

Thrombocytes

The platelet diluting fluid recommended by Baar (1948) was used. This was made up as follows:-

Saponin (BDH)	-	0.25g
Sodium citrate	-	3.5g
Formalin (40% Formaldehyde)	-	1.0ml
Distilled water	to	100.0ml

Baar included 0.1g brilliant cresyl blue but this was found to be unnecessary.

Dilutions were made into white cell diluting pipettes. Blood was drawn up to the 0.5 mark on the pipette and the excess wiped off. Diluting fluid was then drawn up into the pipette until it reached the 11 mark. Pipettes were shaken for several minutes on a mechanical shaker. Counts were carried out in an Improved Neubauer haemocytometer (Hawksley). The first half of the fluid in the pipette was discarded and the chambers filled. The chambers were then placed in a closed box, with moist tissues on the bottom, for 20 minutes before counting.

The red cell counting area was used for counting. Five groups of 16 small squares were counted at 400 times magnification, using a green filter. The count obtained multiplied by 10^9 gave the number of thrombocytes per litre.

Derived red cell indices

These were calculated from the haematology results, using the following formulae:-

Mean corpuscular haemoglobin (MCH) - this is the average haemoglobin content of a single red cell expressed as pico-grams (10^{-12} g)

$$\text{MCH} = \frac{\text{Haemoglobin in g/dl}}{\text{Red blood cells per litre}} \times 10^3$$

Mean corpuscular volume (MCV) - this is the average red cell volume expressed in femtolitres (fl)

$$\text{MCV} = \frac{\text{PCV in l/l}}{\text{RBCs/litre}} \times 10^3$$

Mean corpuscular haemoglobin concentration (MCHC) - this is the concentration of haemoglobin as g/dl of red blood cells.

$$\text{MCHC} = \frac{\text{Haemoglobin in g/dl}}{\text{Packed cell volume in l/l}}$$

Serum Biochemistry and Enzyme Estimations

The methods used for these measurements were those in current use in the clinical laboratory at the Royal (Dick) School of Veterinary Studies, Edinburgh.

In order to reduce costs, the only parameters measured were those considered most likely to reveal changes which would be of clinical relevance in each experiment. This varied with the drug used.

In assessing the results, comparisons were made with figures obtained from normal pigs, performed in the same laboratory if available. Most of these animals were from the same piggery as the experimental pigs. These figures were thought to be more reliable than results quoted by other authors in different parts of the world and from different breeds of pig under different managemental conditions. If control results were not available from this source, other references were consulted.

Total serum protein, determined by the Biuret reaction

Protein forms a coloured complex with cupric ions in alkaline solution. The protein-biuret complex is read against a reagent blank, and the amount of protein

is calculated against a known protein standard carried through the same procedure (Henry, Sobel and Berkman, 1957).

Reagents used were:-

(1) Biuret reagent (Benedict's Qualitative glucose reagent). This was made up as follows:-

(a) Copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), 17.3g, dissolved in 100ml hot distilled water.

(b) Sodium citrate, 173g, and anhydrous sodium carbonate, 100g, dissolved in 800ml water by heating.

When cool (a) was poured into (b) while stirring and diluted to 1 litre at room temperature.

(2) NaOH solution 3% (w/v)

Test-tubes were set up as follows:-

	Blank	Standard	Control	Test
3% NaOH	5.0ml	4.9 ml	4.9 ml	4.9 ml
Protein standard	-	0.1 ml	-	-
Control serum (Commercial)	-	-	0.1 ml	-
Unknown serum	-	-	-	0.1 ml
Biuret reagent	1ml	1ml	1ml	1ml

The tubes were mixed without delay, left at room temperature for at least 15 minutes and then examined for turbidity. If clear, or only a trace was present, the absorbance of standard, control and unknown were read against a reagent blank in a spectrophotometer at 545nm.

Turbidity due to lipaemia was cleared by adding 3ml of ether, shaking vigorously for 30 seconds and centrifuging.

Haemolysis was important because 0.001g Hb/dl is equivalent to 1.9mg serum protein. However Hb concentration of less than 0.1g/dl can be ignored, and haemolysis was seldom as severe as this.

The formula for calculation of total protein in g/litre is
$$\frac{\text{test absorbance}}{\text{standard absorbance}} \times \frac{1000}{0.1} \times \text{g protein in standard}$$

Albumin determination in serum

The method used was similar to that of Dumas, Watson and Biggs (1971). The addition of albumin to a solution of bromocresol green in a 0.075M succinate buffer at pH 4.2 results in an increase in absorbance at 628nm.

Reagents used were:-

(1) Working bromocresol green solution, made by diluting one volume of 0.6mM bromocresol green solution (419mg of bromocresol green in 10ml 0.1N NaOH made up to 1 litre with distilled water) with three volumes of 0.1M succinate buffer (11.9g succinic acid in distilled water, pH adjusted to 4.0 with NaOH and made up to 1 litre with distilled water). A non-ionic surfactant (30% Brij. 35, Fisher Scientific Co.), 4ml/litre, was added to reduce the absorbance of the blank, prevent turbidity and ensure a linear absorbance-concentration relationship, and the pH was adjusted to 4.2.

(2) A standard pig albumin solution, made up in 0.05% aqueous sodium azide. The concentration was determined several times by the total protein method and the average value used as the constant in the calculation. Concentration of Serum albumin in g/litre $\frac{\text{test absorbance}}{\text{standard absorbance}} \times \text{constant}$

Test-tubes were set up as follows:-

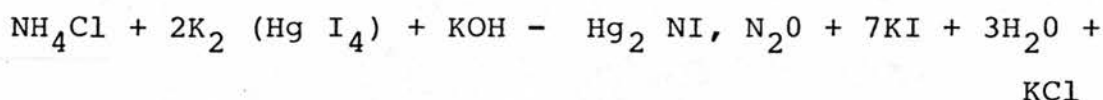
	Blank	Standard	Control	Test
BCG working solution	5.0ml	5.0 ml	5.0 ml	5.0 ml
Albumin standard	-	0.025ml	-	-
Control serum	-	-	0.025ml	-
Test serum	-	-	-	0.025ml

These were allowed to stand at room temperature for 10 minutes and were then read in the spectrophotometer at 628nm against the blank.

Blood Urea Determination

The Urease-Nesslerisation method involves digestion of a sample of blood or serum with urease (obtained from soya beans), converting the urea into ammonium salts. The proteins are precipitated, the supernatant removed and Nessler's reagent added. The colour produced by the ammonium salt and Nessler's reagent is compared colorimetrically with the colour produced under the same conditions by a standard urea solution. Nessler's reagent is an alkaline solution of potassium mercuric iodide. With ammonia this forms dimercuric iodide which gives a yellow colour.

The calculation is:- $\frac{\text{Test}}{\text{Standard}} \times 16 \text{ mmol/l}$



Reagents used were:-

1. 10% Zinc sulphate
2. 0.5N Sodium hydroxide
3. Urea standard - 96mg Urea dissolved in 100ml distilled water (16 mmol/l)
4. Urease suspension - 1 tablet crushed finely and suspended in 5ml of 30% ethanol
5. Nessler's reagent

Test-tubes were set up as follows:-

	Blank	Standard	Control	Test
Distilled water	3.2ml	3.0ml	3.0ml	3ml
Urea standard	-	0.2ml	-	-
Control serum	-	-	0.2ml	-
Blood specimen	-	-	-	0.2ml
Urease suspension	0.2ml	0.2ml	0.2ml	0.2ml

The tubes were incubated in a water bath at 50°C for 15 minutes and then 0.3ml of zinc sulphate and 0.3ml of sodium hydroxide were added to each tube. Each was mixed thoroughly and centrifuged for 10 minutes. During the incubation period the appropriate number of boiling tubes were set up with 5ml distilled water in each and these were cooled in the refrigerator.

After centrifugation, 2ml supernatant was removed

from each tube and added to the water in the boiling tubes. Finally 1ml of Nessler's reagent was added and the yellowish-brown colour produced read immediately against the blank in an EEL (Evans Electroselenium Ltd.) "Spectra" at 460nm.

Serum bilirubin estimation

Methods of detecting and estimating bilirubin in serum are based on the fact that sulphanilic acid diazotised with sodium nitrite and hydrochloric acid, combines with bilirubin in alcoholic solution to form a red-violet azo dye, azo-bilirubin.

In cases of obstructive jaundice, in which mainly conjugated serum bilirubin is increased, the azo-bilirubin colour develops quickly in the absence of alcohol. This is the basis for the direct Van den Bergh reaction, named after the man who demonstrated it. In haemolytic jaundice most of the total bilirubin is in the free state and requires to be brought into solution by the addition of alcohol. This is called the indirect Van den Bergh reaction.

In Van den Bergh's original method, serum proteins were precipitated by ethyl alcohol. Several methods are now available without precipitation of protein. The method of Powell (1944) uses an aqueous solution of sodium benzoate and urea which gives an almost immediate colour development from the whole of the bilirubin present in $1/10$ dilution and is more accurate with low concentrations of bilirubin. Both total and direct

reacting bilirubin can be estimated without precipitation of protein.

Reagents used were:-

(1) Diazo reagent - this was prepared freshly before use by adding 0.3ml of soln B to 10ml of Soln A.

Solution A - 1g of sulphanilic acid dissolved in 15ml concentrated HCl and made up to 1 litre with distilled water.

Solution B - 0.5g sodium nitrite dissolved in distilled water and made up to 100ml.

(2) Diazo blank - 1.5% HCl.

(3) Sodium benzoate - urea solution - 10g sodium benzoate was dissolved in 60ml distilled water, 10g of urea was added and dissolved. The solution was then made up to 100ml. and filtered.

(4) Standard solution of bilirubin: 10mg bilirubin in 100ml chloroform.

Working standard - 4ml stock solution was diluted to 50ml with 95% ethanol.

This equals 0.8mg/100ml bilirubin.

5ml. of standard given above plus 1ml diazo reagent and 4ml of 95% ethanol, gives a colour equivalent to 4mg/100ml if read after 30 minutes. Methyl red artificial standard - 0.29g methyl red was dissolved in glacial acetic acid and made up to 100ml with acetic acid, 1ml of this solution was transferred to a litre flask and 5ml acetic acid plus a little water was added and 14.4g sodium

acetate was washed in and dissolved. This was then made up to 1 litre with distilled water.

1 in 10 dilution = 4mg/100ml bilirubin.

Both total and direct reacting bilirubin can be estimated without precipitation of protein.

Serum and reagents were mixed in four test-tubes as follows:-

	Total bilirubin	Blank	Direct bilirubin	Blank
Serum	0.4ml	0.4ml	0.4ml	0.4ml
Diazo reagent	0.2ml	-	0.2ml	-
Diazo blank	-	0.2ml	-	0.2ml
Benzoate-Urea	3.4ml	3.4ml	-	-
Distilled water	-	-	3.4ml	3.4ml

The tubes were allowed to stand at room temperature for 10 minutes and read against appropriate blanks, at 520nm.

The standard solution was read against a water blank.

(624 green for EEL colorimeter).

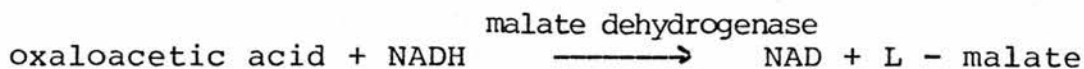
Calculation:- $\frac{\text{Reading of Test}}{\text{Reading of standard}} \times 68.4 = \mu\text{mol of total or direct bilirubin/litre}$

Serum glutamic oxaloacetate transaminase estimation (SGOT)

This was performed using a kit (Calbiochem, California, U.S.A.) using a modification of the method of Karmen, Wroblewski and La Due (1955).

The test principle is

α - ketoglutarate + L-aspartate $\xrightarrow{\text{GOT}}$ L-Glutamate + oxaloacetic acid (GOT - glutamic-oxaloacetate transaminase)



(NAD - Nicotinamide - adenine dinucleotide

NADH - Nicotinamide - adenine dinucleotide reduced)

i.e. α - ketoglutarate and L - aspartate react in the presence of GOT to yield L - glutamate and oxaloacetate. The latter is subsequently reduced by malate dehydrogenase to L - malate and simultaneously, a molar equivalent of NADH is oxidised. The rate of change in absorbance is proportional to the activity of GOT in the sample. Lactate dehydrogenase is present to eliminate endogenous pyruvate, the major source of non-specific side reactions.

The reagents were supplied in "A" and "B" vials, to be reconstituted with distilled water, and mixed before use.

The contents of an "A" vial yields a solution of pH 7.4 containing Phosphate buffer - 0.05 mol/l.

L - aspartic acid - 3.6×10^{-2} mol/l

L - ketoglutarate - 7.6×10^{-3} mol/l

Malate dehydrogenase - 333 iu/l

Lactate dehydrogenase - 333 iu/l

The "B" vial contains the cofactor NADH (2.0×10^{-4} mol/l) and non-reactive stabiliser.

The GOT assay was performed on serum, separated from the clot as soon after collection as possible. It was desirable that the specimens were free from haemolysis. GOT is stable for 3 days at room temperature, for

1 week at 4°C and for 1 month frozen at - 25°C. Activity could still be detected however in samples frozen for 2 years at - 60°C.

Sera of known activity were used as controls.

Using a suitable pipette, 3.0ml of the reagent solution from vial A was dispensed into a clean dry cuvet, with a 1cm light path. The cuvet was placed in a constant temperature waterbath for 3 minutes, at 30°C. The test sample was preincubated for the same length of time in the same bath. To the cuvet was added 0.2ml of the test sample. This was mixed quickly by gentle inversion, avoiding shaking. The cuvet was wiped dry and immediately inserted into the temperature controlled cell compartment of the photometer. Water was used as a blank. The initial absorbance was measured approximately 3 minutes after placing the cuvet in the instrument. Exactly five minutes after the initial reading, the final absorbance was determined. All measurements were made at 340nm.

If the absorbance change of a sample was more than 0.25 in 5 minutes (GOT activity greater than 130 iu/l), or if the sample was icteric or lipaemic, the test was repeated using less sample.

The GOT activity in iu/l = the observed change in absorbance in the 5-minute period $\times 514$.

If less sample was used, the result was multiplied by a dilution factor, depending on the volume of sample.

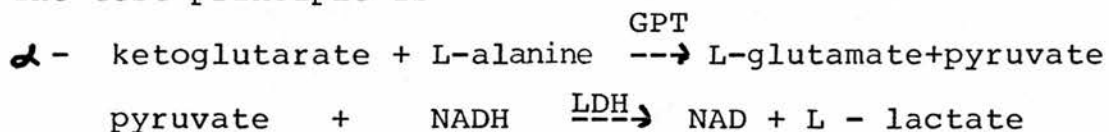
This was 1.94 when 0.1ml of sample was used and 3.81 when 0.05ml was used.

An International Unit is defined as the activity of enzyme which converts 1 micromole of substrate in 1 minute under standard conditions.

Serum glutamic pyruvate transaminase estimation (SGPT)

This was also performed using a kit (Calbiochem) using a modification of the method of Wroblewski and La Due (1956).

The test principle is



In this reaction, α -ketoglutarate and L-alanine react in the presence of GPT to yield L-glutamate and pyruvate. The latter is subsequently reduced by lactate dehydrogenase into L-lactate; simultaneously a molar equivalent of NADH is oxidised. The rate of change in absorbance at 340 nm is proportional to the activity of GPT in the sample.

As with the SGOT kit, the reagents were supplied in "A" and "B" vials to be reconstituted with distilled water and mixed.

The contents of an "A" vial yields a solution of pH 7.4 containing:-

Phosphate buffer - 0.05 mol/l

L - Alanine - 0.17 mol/l

α - ketoglutarate - 7.0×10^{-3} mol/l

Lactate dehydrogenase - 333 iu/l

The "B" vial again contains the cofactor NADH (2.0×10^{-4} mol/l) and non-reactive stabiliser.

The GPT assay was performed on serum as with the GOT assay. Sera of known activity were used as controls.

The method and calculation of results were exactly the same as for the SGOT assay.

The result is also expressed in iu/l.

Serum alkaline phosphatase estimation

This was carried out by the colorimetric method of Bessey, Lowry and Brock (1946).

The principle of the test is as follows:-

p - nitrophenyl phosphate $\xrightarrow{\text{alkaline phosphate}}$ phosphate + p-nitrophenol.

Reagents used were:-

(1) Buffer - glycine buffer - 50 mmol/l pH 10.5

MgCl₂ - 0.5 mmol/l

(2) Substrate - sodium p - nitrophenyl phosphate - 5.5 mmol/l

One tablet of the substrate was dissolved in 10ml of buffer solution.

(3) NaOH solution - 0.02N

Tubes were set up as follows:-

Control blank - 1ml substrate solution Test blank - 1ml substrate solution.

Control test -

1ml substrate solution +0.1ml
control serum

Test -

1ml substrate solution+0.1ml
unknown serum

The samples were added at 30 second intervals exactly. The tubes were incubated at 37°C in a waterbath for 30 minutes.

After this, at 30 second intervals, 10ml NaOH was added and 0.1ml of sera were added to the blanks.

The tubes were mixed well and the liquid poured into cuvetts. The absorbance of each test was read against the blanks at 405 nm in the spectrophotometer.

Alkaline phosphatase (iu/litre) - 200 x absorbance.

Inorganic phosphate determination in serum

This was carried out by the method of Fiske and Subbarow (1925). The principle is as follows:-

The proteins of blood, serum or plasma, are precipitated with trichloroacetic acid. The protein-free filtrate is treated with acid molybdate solution which forms phosphomolybdic acid from any phosphate present. The phosphomolybdic acid is reduced by the addition of 1, 2, 4 - amino-naphthal-sulphonic acid reagent to produce a blue colour whose intensity is proportional to the amount of phosphate present. The molybdenum blue colour is a mixture of the lower oxides of molybdenum.

Reagents used were as follows:-

- (1) 10% trichloroacetic acid.

(2) 10N sulphuric acid - 450ml concentrated sulphuric acid was added to 1,300ml distilled water, taking the necessary precautions to avoid excessive heat being generated. To check, 10ml of this volume was diluted to 100ml with distilled water and a 10ml volume titrated with standard $N/1$ NaOH. Any necessary adjustment was then made.

(3) Molybdate solution - 25g ammonium molybdate was dissolved in 200ml of distilled water, 300ml of 10N sulphuric acid was placed in a litre volumetric flask, the molybdate solution was added and the whole was diluted with distilled water to 1 litre and mixed.

(4) Amino-naphthal sulphonic acid solution (ANSA) 0.2g of 1, 2, 4 -amino naphthal-sulphonic acid, 12g sodium metabisulphite and 2.4g sodium sulphite ($\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$) were dissolved in 100ml of distilled water.

(5) Standard phosphate solution.

Exactly 0.351g of pure dry monopotassium phosphate ($\text{K H}_2\text{PO}_4$) was dissolved in 500ml distilled water in a 1 litre volumetric flask, 10ml of 10N sulphuric acid was added and the solution was diluted to the mark with distilled water. This solution contained 0.00064 millimoles phosphorus in 0.25ml.

Technique -

1ml of serum was added to 4ml of 10% trichloroacetic acid and mixed. This was centrifuged at 3,000rpm for five minutes, 2.5ml of the filtrate was transferred to another tube and test, blank and standard tubes were set up as follows:-

	Blank	Standard	Test	Control
Control serum			-	2.5ml
Protein-free filtrate	-	-	2.5ml	-
10% trichloroacetic acid	2.5ml	2.5ml	-	-
Molybdate solution	0.5ml	0.5ml	0.5ml	0.5ml
Standard solution	-	0.25ml	-	-
A.N.S.A. reagent	0.2ml	0.2ml	0.2ml	0.2ml
Distilled water	1.8ml	1.8ml	1.8ml	1.8ml

The tubes were allowed to stand for 10 minutes for full colour development and the blue colour is read using a red filter ^{at nm} / 608 in an EEL colorimeter.

$$\text{Calculation} - \frac{\text{Reading of test}}{\text{Reading of standard}} \times \frac{0.00064(a)}{1} \times \frac{1000}{0.5(b)} = \frac{T}{S} \times 1.292$$

$$= \text{mmol inorganic phosphate/litre}$$

N.B. a) = amount of standard used

b) = amount of test used.

Serum Calcium and Magnesium

Calcium and magnesium were measured by atomic absorption spectrophotometry. This is an analytical technique based on the absorption of photons by an atomic vapour. The wavelength at which absorption occurs is characteristic of the element and the degree of absorption is a function of the concentration of atoms in the vapour.

To prepare the samples, 0.2ml of serum was diluted to 10ml (i.e., $\frac{1}{50}$) with 0.1% lanthanum chloride in a volumetric flask.

Standard calibration solutions were prepared as follows. The low standard for calcium was 0.625 mmol/litre, made by diluting 1.25ml of stock 2.5mmol/litre calcium chloride solution with 0.1% lanthanum chloride to 250ml. The high standard was 3.125mmol/litre, made by diluting 6.25ml of stock solution as above. The low standard for magnesium was 0.4mmol/litre made by diluting 4.8ml of 0.416mmol/litre magnesium chloride solution to 250ml as above. The high standard was 1.6mmol/litre made by diluting 19.23ml of stock solution as above.

The wave lengths were 422.7 nm for calcium and 285nm for magnesium. The instrument used was Model A3400 (Shandon Southern). This consists of a light source, which is a hollow cathode lamp, a nebulizer and air/acetylene burner for sample atomization, a wavelength selector, a photomultiplier detector and an amplifier and read-out system.

Serum sodium and potassium

These were measured by flame emission spectrophotometry. This technique measures the light emitted when an element, which has been heated and atomised in a flame and has absorbed energy, returns to a lower energy level. This emitted light has a wavelength characteristic of the element.

The same instrument was used as for atomic absorption spectrophotometry, the Shandon Southern A3400

but using the emission mode.

Serum samples were prepared by diluting with distilled water to $1/100$ for potassium estimation and $1/500$ for sodium estimation.

Calibration solutions were made by dissolving 7.46g of pure potassium chloride and 58.5g of pure sodium chloride in distilled water and making up to 1 litre (1000mmol/l) respectively.

The wavelength for potassium was 766.5nm and for sodium 589nm.

Serum Chloride

This was measured using an EEL chloride meter (Evans Electroselenium Ltd.). The principle of the method is as follows.

A known current flow between two silver electrodes is by definition the transfer of a known number of silver ions. When such a transfer takes place through an aqueous solution of chloride, the two substances combine, ion for ion, until all chloride has been precipitated as silver chloride. This stage is marked by a change in the conductivity of the solution and the appearance of free silver ions. In the chloride Meter this change is registered by two sensing electrodes and applied to stop a timer calibrated to give a direct measurement.

The sample used was 0.2ml serum.

A standard sodium chloride solution was made by

dissolving 3.946g in de-ionised water and making up to 500ml.

The acid buffer solution was made by adding 46ml glacial acetic acid and 3ml concentrated nitric acid to de-ionised water and making up to 1 litre.

Gelatine solution was made by adding 2.5g gelatine to 250ml de-ionised water, bringing to the boil and adding slowly 0.5g thymol blue in 100ml methanol. When cold 0.5g thymol was added as a preservative.

The test sample was added to 13ml acid buffer and five drops of gelatine solution, and was read against a blank of buffer and gelatine solution only.

The serum biochemistry estimations, with the exception of blood urea, were carried out by Mr. R. Brown and the technical staff of the Department of Medicine, Royal (Dick) School of Veterinary Studies, Field Station, Easter Bush, Roslin.

Pathology

Autopsy examinations were carried out as soon as possible after death, usually within 20 minutes after euthanasia, by Mr. K.W. Head, Department of Veterinary Pathology, Royal(Dick) School of Veterinary Studies, Edinburgh. I was present at all autopsies and assisted where possible. The autopsy results documented in this thesis were compiled from Mr. Head's detailed records, and are included because it would have been difficult to interpret the clinical and haematological findings without also considering the post-mortem findings.

The lymph nodes, thymus, spleen, liver, kidneys and adrenal glands were dissected out and weighed. Mean values were obtained for kidneys and adrenals but only one of a pair of lymph nodes, always from the same side, was weighed.

The anatomy of the lymph node in the pig is unusual. The tissues which correspond functionally with the medulla and paracortical zone in other mammalian species surround and are interspersed with the lymphoid nodules. The terms "cortex" and "medulla" are used in the autopsy reports to describe the regions of the node whose functions correspond with the cortex and medulla of other species. They do not refer to position within the node.

CHAPTER I
PREDNISOLONE

INTRODUCTION

In 1937, Selye described a syndrome, which occurred following exposure of an organism to various harmful or potentially harmful agents, and which was characterised by adrenocortical hypertrophy and increased secretion of adrenal corticosteroid hormones. This became known as the "stress syndrome", and Selye's observations led to widespread interest in, and study of, the adrenal cortex and the role of the hormones which it produces. In the same year, Moon (1937) showed that growth in castrated rats could be inhibited with pituitary extract.

Several years later, Dougherty and White (1943a and b and 1944) showed that injection of adrenocorticotrophic hormone (ACTH) into normal mice was associated with loss of weight of axillary, inguinal and mesenteric lymph nodes and of the thymus, a decrease in total white blood cell count, a lymphocytopenia and a polymorphonuclearleucocytosis. Similar findings were obtained when mice were injected with adrenal cortical hormone. Soon after this, Murphy and Sturm (1944) showed that ACTH administration was followed by rapid regression of transplanted murine lymphoid tumours, and Heilman and Kendall (1944) achieved similar results with 11-dehydro-17-hydroxycorticosterone, which they called "Compound E". They found that the tumour recurred after the treatment was discontinued and that it was then much less responsive

to the hormone than it had been originally. Law and Speirs (1947) found that when adrenocortical extract was injected into mice in the terminal stages of spontaneous lymphoid leukaemia, there was a decrease in circulating lymphocytes, extensive degeneration of immature lymphocytes, and a decrease in the size of lymph nodes and the thymus.

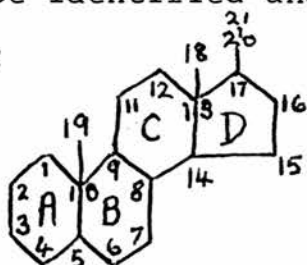
The first report of the effects of ACTH and cortisone on lymphoid tumours in man was that of Pearson, Eliel, Rawson, Dobriner and Rhoads (1949). They found that there was a dramatic decrease in size of the liver, spleen and lymph nodes, though remissions were usually incomplete and the disease recurred when treatment was discontinued. They described problems with side effects such as fluid retention. In another early trial, Rosenthal, Saunders, Schwartz, Zannos, Santiago and Dameshek (1951) observed excellent tumour responses, but also stated that they were of short duration.

In 1954, it was discovered that it was possible to increase the therapeutic efficacy of cortisone by inducing modifications in the steroid molecule. By a synthetic process, 9 α -fluorocortisol was produced and was found to have increased anti-inflammatory effect compared with cortisone (Myles and Daly, 1974). Research was therefore directed to the production of new corticosteroids.

Good responses in malignant lymphomas and lymphoid leukaemias, using massive doses of the closely related synthetic corticosteroids prednisone and prednisolone, were reported by Hill, Marshall and Falco (1956) and Ranney and Gellhorn (1957). Further trials using corticosteroids in the treatment of lymphomas were reviewed by Kyle, McParland and Dameshek (1962), Hall, Choi, Abadi and Krant (1967) and Ezdinli, Stutzman, Aungst and Firat (1969), in addition to describing their own observations. As a result of these trials, it was generally accepted that corticosteroids, especially prednisolone and prednisone, were of value in the treatment of lymphoid tumours in man.

The effects that the corticosteroids have in these diseases are mainly due to their action on lymphoid cells, though they also have important effects on other tissues (See Page 119)

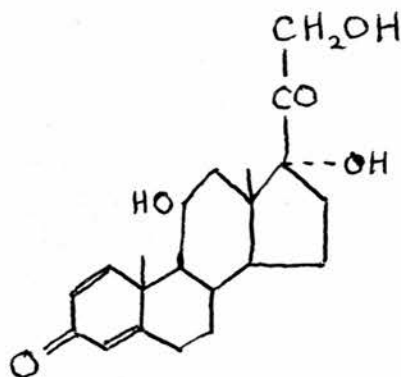
All steroid hormones have the same basic structure. The nucleus consists of three joined six-carbon rings linked to a five-carbon ring. Each carbon atom has been given a number so that the position of substituted groups can be identified and the rings are identified by a letter:



The naturally-occurring corticosteroid cortisol, or hydrocortisone, corticosterone and certain of their synthetic analogues, are referred to as glucocorticosteroids because of their action of increasing hepatic glucose output. They stimulate hepatic gluconeogenesis, while depressing protein synthesis in muscle.

Dougherty, Berliner, Schneebeli and Berliner (1964) summarized the characteristics of glucocorticosteroids which are active on lymphoid tissue. They have an unsaturated A ring, a ketone at position 3, an O or OH group at position 11 and a COCH_2OH group filling positions 20 and 21.

Prednisolone conforms to this description, having the following structure:-



Two main types of lymphocyte populations are recognised, those which mature in the thymus known as "T" cells, and those which mature in other sites, known as "B" cells. T cells are involved with cell-mediated immune responses, and graft rejection. B cells are precursors of plasma cells which produce immunoglobulins.

There are two very important factors which determine the effects of corticosteroids on lymphoid tissue (Claman, 1972).

Firstly, there are species differences in susceptibility to corticosteroids. The basis of these differences is still not understood, but certain species have been defined as steroid-sensitive and others as steroid-resistant, depending on the ease with which lymphoid depletion is produced after a given regimen of systemic glucocorticosteroids.

Secondly, there are differences in sensitivity between different populations of lymphoid cells within the same species, and in addition, corticosteroid effects on malignant lymphoid cells differ from their effects on normal cells. This was demonstrated by Schrek (1961) using human and rat lymphocytes.

In steroid-sensitive species such as the mouse, rat, hamster and rabbit (Claman 1972), administration of corticosteroids produces rapid involution of the thymus, shrinkage of spleen and lymph nodes, lymphopenia, inhibition of antibody production and graft rejection inhibition. Normal lymphocytes from these species are killed by culturing them with low concentrations of corticosteroids.

On the other hand, lymphocytes from steroid-resistant species such as the ferret, guinea-pig, monkey and man are much more difficult to lyse in vitro even with much

higher concentrations of corticosteroid. Malignant lymphocytes are much more easily destroyed, however. In vivo, some involution of the thymus occurs but only after several days of high dosage. In man, a decrease in blood lymphocyte counts may occur, soon after dosing, but it is probable that this is due to redistribution of lymphocytes, to spleen and bone-marrow, rather than destruction. In leukaemia, however, a rapid fall in blood lymphocytes may be accompanied by a rise in serum uric acid, which strongly suggests that malignant lymphocytes are killed in vivo by corticosteroids, as well as in vitro. (Claman, 1972).

Other haematological effects in man have been widely reported. A neutrophil leucocytosis usually occurs, especially pronounced in children and monocytosis may also occur (John, 1966). The neutrophilia appears to be due to both an influx of neutrophils from the bone marrow and retention within the blood as a result of decreased capillary permeability (Myles, and Daly/¹⁹⁷⁴). The effect occurs immediately after corticosteroid administration is started and persists throughout treatment. Depletion of circulating monocytes also occurs (Fauci and Dale, 1974). The eosinophil count falls rapidly (Thorn, Forsham, Prunty and Hills, 1948) and later there is a less marked fall in circulating basophils (Juhlin, 1963). The effect of corticosteroids on thrombocyte counts appears to be variable. Forsham, Thorn, Prunty and Hills (1948)

in early studies with ACTH reported no change in thrombocyte counts. Cohen and Gardner (1961) reported depression of bone marrow thrombocyte production during corticosteroid therapy of patients with idiopathic thrombocytopenic purpura, and Hall et al., (1967) reported the development of thrombocytopenia in two patients with lymphomas treated with corticosteroids.

However, Rosenthal et al., (1951), Kyle et al., (1962) and Burningham, Restrepo, Pugh, Brown, Schlossman, Khuri, Lessner and Harrington (1964) reported striking improvements in thrombocyte counts in patients with malignant lymphoproliferative diseases.

According to Myles and Daly (1974), corticosteroids do not produce any effects on erythropoiesis or circulating red cells in normal humans. However, Fisher (1958) showed that red cell parameters in normal rats were significantly increased by administration of corticosteroid, and reviewed the reported evidence supporting the theory that adrenocortical hormones do influence red cell production. Agarwal (1964) reported that haemoglobin and total red cell volume were increased in asthmatic children treated with corticosteroids. Beneficial effects on the red cell picture have been reported by many authors in collagen diseases, anaemias and lymphoid malignant diseases during corticosteroid therapy.

The mechanisms of action of the glucocorticosteroids at the cellular level are still not fully understood. They affect the metabolism of most tissues and frequently,

117
 Schopshire
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they influence a number of functions in the same tissue, which together produce a pattern of metabolic changes. Corticosteroids, whether produced naturally by the adrenal cortex, or administered, are soluble in plasma to a certain extent, but also tend to bind with protein, mostly with a specific alpha globulin, known as "transcortin" or "corticosteroid-binding-globulin", or, to a much lesser extent, with other serum proteins such as albumin. The resulting protein-bound complex acts as a circulating reservoir. Only free corticosteroid, which is in equilibrium with the bound corticosteroid, is active and capable of entering cells. The synthetic corticosteroids have less affinity for transcortin than naturally occurring cortisol, and this accounts for their increased potency.

After diffusion through the cell membrane, corticosteroids bind to specific protein receptors in the cytoplasm. Then the steroid-receptor complex enters the nucleus and is thought to bind to specific sites on the chromatin, possibly to DNA itself, where it may modify transcription of DNA into RNA, influencing either the amount or activity of messenger RNA. The effects on cell metabolism may be either catabolic or anabolic depending on the tissue. The catabolic effects on lymphoid tissue may lead to cell death or to growth inhibition. The mechanisms of action of the glucocorticoids were discussed in detail by Baxter and Forsham (1972).

Until recently, it was widely believed that the steroid-specific cytoplasmic receptor interaction was necessary for a response, and therefore the sensitivity of tissues, in particular malignant lymphoid tissues, could be predicted by estimating the steroid binding capacity (Lippman, Halterman, Leventhal, Perry and Thompson, 1973). However, Crabtree, Smith and Munck (1978) and Duval and Homo (1978) stated that it was not possible to correlate cytoplasmic receptor levels with glucocorticoid response.

Apart from their effects on lymphoid tissue, corticosteroids have many other actions, some of which are clinically desirable and others which make their use hazardous.

These actions were discussed in detail and the extensive literature reviewed by David, Grieco and Cushman (1970) and Myles and Daly (1974), the most important being as follows:-

a) Anti-inflammatory action. Corticosteroids are widely used clinically for this effect, particularly in cases of arthritis and skin disease. Vascular permeability is decreased, and neutrophil and macrophage migration towards areas of inflammation are inhibited. Fibroblast proliferation is also inhibited and collagen deposition decreased. It is also likely that interferon synthesis may be impaired. When these effects

are combined with the previously discussed actions on lymphoid tissue, it is not surprising that there is increased susceptibility to virus, bacterial and fungal infections. Infections are particularly common in patients with neoplastic diseases when high doses of corticosteroids are used and immunosuppression is a feature of the condition.

Another result of the anti-inflammatory action may be a delay in wound-healing.

(b) Corticosteroid therapy in man is associated with an increased incidence of gastric ulcers, particularly in children, in whom gastric ulcers are normally very uncommon. Possible mechanisms are increased acid secretion in response to histamine stimulation, reduction in quantity of gastric mucus and changes in its quality, and reduction in turnover of gastric epithelial cells. The evidence suggests that the action on the stomach is systemic rather than local.

(c) The glucocorticosteroids cause increased hepatic gluconeogenesis and glycogen deposition and tend to produce hyperglycaemia, with the possibility of precipitating, or aggravating existing diabetes mellitus.

(d) There is inhibition of protein synthesis and a catabolic effect on muscle protein. Myopathy, resulting in muscular weakness, is a common side-effect of corticosteroid therapy but histological changes in muscles are slight and the condition is reversible when therapy is reduced or withdrawn.

(e) There are alterations in fat distribution with corticosteroid therapy. In man, total body fat increases, though this may be partly due to an increase in dietary intake, because there is also an improvement in appetite.

(f) Electrolyte metabolism is commonly affected, although the extent of this varies with the type of corticosteroid used. Sodium retention and potassium loss may occur, though the synthetic glucocorticosteroids have less salt-retaining effect than cortisol. Calcium transport across the gut wall is decreased, possibly as a result of antagonism of the calcium absorption effect of Vitamin D, and there may be a decrease in renal proximal tubular reabsorption of calcium and magnesium. Hypophosphataemia and phosphaturia may occur.

(g) Polydipsia and polyuria are fairly common side-effects in man. However, large doses of corticosteroids cause water retention which may result in problems with oedema, hypertension or even cardiac failure.

(h) Osteoporosis often occurs during corticosteroid therapy, producing vertebral collapse and an increased tendency for other bones to fracture. Both increased bone resorption and reduced new bone formation have been reported. Osteoporosis may take years to develop, and x-ray changes are not apparent until the condition is advanced.

(i) Long-term corticosteroid therapy in children is often associated with growth retardation, especially when

Always^{P122}
given
daily
in leukaemia
& lymphoma,
though is
short courses

Good
prevent
of steroid
effects.

administered daily. Intermittent therapy is less likely to cause the problem. When the drug is withdrawn a spurt of growth often occurs.

(j) Suppression of the hypothalamic-pituitary-adrenal system (HPA). This is the negative feedback system which controls the release of ACTH from the pituitary and in turn the conversion of cholesterol to cortisol in the adrenal cortex. If doses of corticosteroids are administered which are higher than the physiological range, the inhibitory feedback pathway is activated. Adrenal atrophy is apparent in many species after 10 days of high-dose corticosteroid therapy (Melby, 1974).

Sudden withdrawal of corticosteroid therapy is dangerous, because, especially in stressful situations, normal HPA function and production of natural corticosteroid hormones cannot recover rapidly enough to replace the administered corticosteroid, and adrenal failure may result. Alternate day or intermittent therapy does not suppress the HPA system to the same extent, and daily therapy is now avoided if at all possible. The reasons for this were discussed by Melby (1974). Burningham et al., (1964) reported successful results in leukaemias and lymphomas with high-dose weekly corticosteroid therapy and stated that side-effects with this protocol were minimal.

Many other side-effects of corticosteroid treatment

have been reported but those mentioned above are the commonest in man.

Bearing in mind the information gained from the literature, trials were designed to investigate the effects of the commonly used corticosteroid prednisolone in normal and lymphosarcomatous pigs.

EFFECTS OF PREDNISOLONE ON NORMAL PIGSMATERIALS AND METHODS

For the first trial, eight normal pigs were used. They were all littermates and there were five females and three castrated males. The sire and dam were of the lymphosarcoma strain but were both non-carriers. The piglets were weaned at 8 weeks, and housing, management and feeding were as described in the General Methods section.

They were randomly allotted to four groups, of two pigs in each - low-dose, medium-dose, high-dose and controls.

Pre-treatment procedures (See General Methods)

At weekly intervals, from 3 weeks before treatment commenced, all the pigs were weighed and blood-sampled. EDTA samples were taken for WBC and RBC counts, PCV, Hb, differential and platelet counts.

Sensitisation with DNFB was performed 11 days before treatment started. The pigs were observed in their pens daily for appetite and general fitness and state of urine and faeces.

Treatment procedure

The prednisolone preparation used was prednisolone sodium phosphate, 16 mg/ml (Codelsol, MSD).

The pigs were 12 weeks old at the start of treatment. The dose levels used were as follows:-

Pig no. 1)	GROUP A	-	2 mg/kg
Pig no. 6)			
Pig no. 5)	GROUP B	-	4 mg/kg
Pig no. 8)			
Pig no. 3)	GROUP C	-	6 mg/kg
Pig no. 7)			
Pig no. 2)	GROUP D	-	Saline equal in volume to the medium dose.
Pig no. 4)			

The drug, or saline, was administered by intramuscular injection after swabbing the site with spirit-moistened cotton wool. Four injection sites were used in rotation i.e. the right and left hind legs and the right and left sides of the neck. The first injections were administered immediately after the last pre-treatment blood sample, and the pigs were injected once daily for 5 days per week. Doses were adjusted for weight gain.

Treatment was continued for 5.5 weeks. During this time, the pigs were examined clinically and rectal temperatures recorded daily, at the time of each injection. Injections and temperature recording were always carried out at the same time each day. Weekly weighing and blood sampling were continued. In addition, sera from clotted samples were taken to measure serum sodium, potassium, chloride, calcium, magnesium, inorganic phosphate, total protein and albumin.

DNFB skin test were carried out at 2 weeks, 3 weeks, 4 weeks and 5 weeks after treatment started. The first test was unsatisfactory because 1% DNFB was tried and found to be too weak. The concentration used at 3 and 4 weeks was 10%, and at 5 weeks was 5%.

After 5½ weeks of treatment, one pig from each pair was killed by intravenous pentobarbitone overdose, and autopsies were carried out as soon as possible.

Treatment of the remaining animals continued until the end of that week, being reduced to half, quarter and one-eighth during the last three days before being finally discontinued. Detailed clinical examinations were stopped but the pigs were observed daily in the pen.

Skin tests were carried out again 2 weeks and 5 weeks after treatment was stopped with 10% DNFB.

After five weeks, when it was considered that the visible effects of the drug had disappeared, the animals were slaughtered and autopsied.

RESULTS

Clinical Examinations (For Clinical records, see Tables 1 to 8
Appendix I)

Throughout the experiment, all the pigs were bright and active. Appetites were excellent, but since the pigs were not allowed ad lib feeding, and individual intakes could not be measured, it was not possible to detect any change in appetite. However, it did appear that the pigs drank more frequently from their automatic water bowls during the treatment period and also that they passed more urine, as the floor of the pen became very wet. These observations were subjective, because facilities were not available for measuring volume of water intake and urine output. Pigs on the higher dose levels acquired a markedly "pot-bellied" appearance after the first two or three weeks of treatment, and pigs 3, 5 and 8 tended to show rectal eversion occasionally from the 3rd week of treatment onwards. This corrected itself spontaneously.

Only one animal had diarrhoea during the experiment. This was pig 2, a control, in the week before the start of dosing. This animal was the "runt" of the litter, in poorest condition from the beginning. The diarrhoea cleared up without treatment and did not recur.

Rectal temperatures varied widely during the experiment, even though recorded at the same time each day in animals which showed no evidence of fever (See Table 4).

PREDNISOLONE IN NORMAL PIGS - A RANGE OF RECTAL TEMPERATURES (°C) RECORDED PER WEEK

GROUP	PIG NUMBER	CONTROLS														MEDIUM DOSE - 4 mg/kg							HIGH DOSE - 6 mg/kg						
		2		4		1		6		5		8		3		7													
		MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX						
WEEK-1	RANGE	39.2	40.2	39.6	40.1	39.3	40.4	39.5	40.0	38.9	40.5	38.5	39.5	39.0	39.7	39.5	39.8												
WEEK 1		39.8	39.2	39.1	39.5	39.6	39.9	39.1	40.1	39.7	40.0	38.8	39.7	39.2	40.0	39.6	40.0												
WEEK 2		38.9	40.1	39.0	39.4	39.9	39.5	39.8	39.9	39.2	40.1	39.0	39.7	39.0	39.7	39.5	40.1												
WEEK 3		38.8	39.9	38.7	39.4	39.3	40.1	39.2	40.0	38.6	39.9	38.7	39.5	39.2	39.5	39.3	39.9												
WEEK 4		39.2	39.7	39.4	39.6	38.7	40.0	39.7	39.8	39.5	40.0	38.8	39.5	38.9	39.4	39.2	39.7												
WEEK 5		38.8	39.3	39.2	39.5	39.0	39.4	39.2	40.0	39.2	40.1	38.5	39.3	38.7	39.2	39.5	39.6												
WEEK 6		38.8	39.5	38.9	39.5	39.1	39.2	39.3	40.0	39.5	39.8	38.1	39.6	38.1	39.1	38.7	39.2												
		END OF TREATMENT																											

No pattern could be established, but excitement caused by handling probably accounted for some of the highest temperatures recorded.

TABLE 5
BODY WEIGHT GAINS - PREDNISOLONE IN NORMAL PIGS

Pig No.	Dose Level	Wt. at start of treatment (kg)	Wt. at end of treatment (kg)	Gain	Wt. at end of expt. (kg)	Gain	Total Gain
3	6mg/kg	17	25.5	8.5	40	14.5	23
7	"	16	23.5*	7.5	-	-	-
5	4mg/kg	18	29*	11	-	-	-
8	"	21	33	12	52.5	19.5	31.5
1	2mg/kg	17	27*	10	-	-	-
6	"	13	22	9	40	18	27
2	Control	13	22*	9	-	-	-
4		16	30.5	14.5	49	18.5	33

* Destroyed at end of treatment.

Weight Gains

From Table 5 , the following conclusions can be drawn:-

- a) All pigs not destroyed at the end of treatment gained more weight during the post-treatment period than during the treatment period, even the control animal. This could have been due to recovery from the stress of weaning and the infections to which young pigs are susceptible at this time, and also reduced handling stress.
- b) Both high-dose pigs, 3 and 7, had lower weight gains during the treatment period than any of the other pigs, despite the fact that their commencing weights were average for the litter.
- c) Pig 3 also had the lowest weight gain during the post-treatment period.
- d) Control pig 4 had the best total weight gain (33kg). Control pig 2 was in poor condition, as previously mentioned, therefore it would be unlikely to show a similar weight gain. However its weight gain was still greater than those of the high-dose pigs.

It would appear, therefore, that treatment with prednisolone at the 6mg/kg dose level resulted in depression of growth.

DNFB Skin Test Results

The reaction scores in Table 6 suggest that by Week 5 of the treatment both high-dose group pigs (3 and 7), one medium-dose pig (5) and one low-dose pig

TABLE 6

PREDNISOLONE IN NORMAL PIGS

DNFB Skin Tests Results

Pig No.	Dose Level	PRE-TREATMENT At sensitisation	DURING TREATMENT				POST-TREATMENT		
			Week 2*	Week 3	Week 4	Week 5 ⁺	Week 8	Week 11	
3	6mg/kg	3	0	3	3	1	1	4	
7	"	1	0	2	2	0	Dead	-	
5	4mg/kg	0	0	1	3	1	Dead	-	
8	"	4	0	3	3	3	0	4	
1	2mg/kg	3	0	3	3	0	Dead	-	
6	"	3	0	2	3	3	2	4	
2	Control	0	0	2	4	4	Dead	-	
4	"	4	0	3	3	3	0	4	
			*1% DNFB				+5% DNFB		

(1) showed an anergy to DNFB, compared with the controls. This could not have been due to using a weaker solution of DNFB because the controls had the same scores during Week 4, when the stronger solution was used, as they did in Week 5. After withdrawal of the drug, control pig 4 had an unexpectedly poor response in Week 8 and a negative response was also recorded at this time in medium-dose pig 8. However, by Week 11, the remaining control and treated pigs all had similar strong reactions. It would appear, therefore, that treatment with prednisolone depressed cell-mediated response, particularly in those pigs given the highest dose.

Haematology

See Tables 9 to 24 Appendix I.

White blood cell counts were high in all groups during the pretreatment and treatment periods. This was due mainly to the neutrophil counts which were elevated compared with figures from other normal pigs in this piggery, particularly in the Group A pigs 1 and 6, Group C pig 7 and control pig 2. Mean \pm 1 S.D. of WBC and absolute neutrophil, lymphocyte and monocyte counts are shown in Table 7. Lymphocyte counts were also high as is to be expected at from 9 to 16 weeks of age (McTaggart, 1975).

During the treatment period, WBC counts in the treated groups fell slightly compared with the control group, though standard deviations were wide. Absolute

TABLE 7.

PREDNISOLONE IN NORMAL PIGS																		
GROUP D - SALINE - TREATED CONTROLS				GROUP A - LOW DOSE				GROUP B - MEDIUM DOSE				GROUP C - HIGH DOSE						
WBC - MEANS \pm 1 S.D. ($\times 10^9$ /L)				T. NEUTROPHILS - MEANS \pm 1 S.D.				LYMPHOCYTES - MEANS \pm 1 S.D				MONOCYTES - MEANS \pm 1 S.D						
DAYS FROM START OF DOSING		GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C	
- 12		27.9 \pm 1.0	26.6 \pm 3.2	33.0 \pm 4.1	34.1 \pm 15.1	11.2 \pm 2.0	14.5 \pm 3.0	18.2 \pm 3.7	18.9 \pm 11.2	15.3 \pm 1.8	10.4 \pm 0.1	13.5 \pm 1.2	13.3 \pm 0.6	1.4 \pm 0	0.7 \pm 0.1	1.4 \pm 0.6	1.4 \pm 0.5	
- 5		28.5 \pm 9.2	34.2 \pm 4.3	28.8 \pm 4.5	35.0 \pm 15.6	10.5 \pm 3.0	19.9 \pm 4.4	11.3 \pm 4.0	21.5 \pm 14.5	15.4 \pm 3.4	11.9 \pm 0.1	15.1 \pm 1.8	11.6 \pm 0.5	2.2 \pm 0	2.2 \pm 0.9	1.5 \pm 0.5	1.5 \pm 0.6	
0		30.8 \pm 10.1	35.9 \pm 0.4	26.3 \pm 7.2	30.1 \pm 9.0	16.2 \pm 9.8	20.7 \pm 2.0	9.0 \pm 3.2	15.6 \pm 9.9	11.9 \pm 0.8	12.2 \pm 0.1	14.6 \pm 2.9	11.5 \pm 1.8	2.7 \pm 1.7	2.5 \pm 0.9	2.3 \pm 0.9	2.3 \pm 0.9	
Start of Dosing																		
7		31.2 \pm 9.5	30.7 \pm 1.0	28.3 \pm 3.2	29.3 \pm 9.5	15.5 \pm 7.6	17.4 \pm 2.3	11.6 \pm 0.7	14.5 \pm 6.3	11.6 \pm 1.4	11.6 \pm 2.0	14.2 \pm 2.5	11.8 \pm 2.4	1.4 \pm 0.5	2.4 \pm 0.2	2.8 \pm 0.7	2.8 \pm 0.7	
14		32.7 \pm 8.2	28.3 \pm 2.7	26.8 \pm 3.5	30.3 \pm 6.4	16.1 \pm 9.1	16.9 \pm 1.1	12.0 \pm 4.6	15.8 \pm 5.9	13.2 \pm 2.9	9.1 \pm 0.8	12.5 \pm 1.3	12.3 \pm 1.1	1.9 \pm 0.7	1.9 \pm 0.2	1.9 \pm 0.5	1.9 \pm 0.5	
21		31.6 \pm 15.3	27.2 \pm 1.5	23.7 \pm 0.4	27.4 \pm 6.3	17.1 \pm 12.4	12.9 \pm 2.5	7.7 \pm 1.0	12.8 \pm 5.0	10.9 \pm 1.1	10.7 \pm 1.4	13.1 \pm 0.9	11.1 \pm 0.3	0.6 \pm 0.1	0.5 \pm 0.1	0.5 \pm 0.1	0.5 \pm 0.1	
28		28.2 \pm 12.2	20.8 \pm 1.3	20.3 \pm 2.3	21.7 \pm 6.9	14.7 \pm 10.2	9.9 \pm 2.4	7.0 \pm 0.6	9.1 \pm 3.6	10.7 \pm 0.7	8.1 \pm 0.3	11.1 \pm 2.4	10.0 \pm 2.9	2.1 \pm 1.2	1.6 \pm 0.1	1.6 \pm 0.2	1.6 \pm 0.2	
35		24.4 \pm 5.7	20.6 \pm 1.3	18.6 \pm 0.3	13.2 \pm 6.4	11.5 \pm 6.2	8.7 \pm 2.0	5.7 \pm 1.2	8.5 \pm 4.4	10.3 \pm 0.1	9.3 \pm 0	10.0 \pm 1.4	6.6 \pm 0.6	2.1 \pm 0.4	2.3 \pm 0.2	2.0 \pm 0.7	2.0 \pm 0.7	
End of Dosing																		
42		18.9	17.8	15.1	13.2	7.7	7.6	6.9	6.1	9.4	8.1	6.9	5.4	1.9	0.9	1.6	1.6	
49		21.7	18.8	20.0	16.1	9.1	8.0	7.9	8.8	9.8	7.6	10.2	5.8	1.6	1.8	1.2	1.2	
56		20.4	14.3	17.6	12.0	9.7	5.2	9.0	5.3	9.0	8.6	7.6	5.3	1.1	0.8	1.4	1.4	
63		15.4	14.4	17.8	11.6	5.9	6.5	6.9	4.6	8.2	5.2	8.8	5.2	1.9	1.4	1.5	1.5	
70		18.9	15.1	15.1	11.5	6.5	6.7	5.5	5.1	10.6	6.5	8.1	5.0	1.4	1.1	1.3	1.3	
Σ		One animal in each group only																

neutrophil counts fell in all groups, as did the lymphocyte counts, but the fall in lymphocyte counts was greater in the treated groups than in the control group. If the differences between absolute lymphocyte counts at the beginning and end of treatment are considered for each pig, it appears that the effect is dose-related. See Table 8.

TABLE 8

EFFECT OF PREDNISOLONE ON ABSOLUTE LYMPHOCYTE COUNTS
x $10^9/L$

Dose Level	Control		2 mg/kg		4 mg/kg		6 mg/kg	
Pig No.	2	4	1	6	5	8	3	7
Pre-treatment	11.4	12.5	12.3	12.1	12.5	16.6	12.8	10.2
End of treatment	10.2	10.3	9.3	9.2	9.0	10.9	6.2	7.0
Difference	1.2	2.2	3.0	2.9	3.5	5.7	6.6	3.2

Monocyte counts varied considerably throughout the experiment and there were no differences between groups which could be attributed to the treatment.

After the treatment was stopped, absolute neutrophil counts in the remaining animals continued to fall to more normal levels, and there were no differences between the pigs from each treatment group. Absolute lymphocyte counts remained lower in the previously treated pigs than in the control animal, especially in the pig which had received the highest dose.

TABLE 9

PREDNISOLONE IN NORMAL PIGS - ABSOLUTE EOSINOPHIL COUNTS $\times 10^9/L$

DAYS FROM START OF DOSING	CONTROLS		2 mg/kg		4 mg/kg		6 mg/kg	
	2	4	1	6	5	8	3	7
- 12	0.29	0	0	0	1.20	0	0	0.45
- 5	0	0.22	0	0	0.26	0	0.24	0.46
0	0.38	0.24	0.36	0	0	0.31	0.24	0.73
Start of Dosing								
7	0.19	0.12	0.16	0.15	0	0	0	0.18
14	0	0.40	0.30	0.26	0.37	0.44	0.39	0.17
21	0.21	0.31	0.52	0.56	0.35	0.12	0.57	0.80
28	0	0.20	0.60	0.43	0.93	0.11	0.25	1.06
35	0	0.41	0.59	0.11	0.94	0.09	0.27	1.36
End of Dosing								
42	Dead	0.19	Dead	0.09	Dead	0.30	0.13	Dead
49		0.65		0.38		0.10	0.32	
56		0.20		0.29		0.18	0.06	
63		0.46		0.29		0.53	0.29	
70		0.47		0.45		0.38	0.17	

Eosinophil counts and basophil counts remained at low levels throughout the experiment and therefore any effects are difficult to assess. However, absolute eosinophil counts, calculated from differential counts, are shown in Table 9. In all the treated animals, there was an increase in eosinophil counts during the treatment period, compared with the last pretreatment counts, and at the end of treatment, three out of the six treated animals, one from each dose group, had higher eosinophil counts than either of the control pigs. Therefore it appears that prednisolone treatment did not depress eosinophil counts in these animals.

Table 10 shows mean \pm 1 S.D. of RBC counts, PCV and Hb For figures from individual pigs, see Appendix

I. Table 10 shows that from day 0 to day 35, the red cell counts of the control pigs changed little and their PCV and Hb levels increased only slightly. Although standard deviations are wide it appears that there was a greater increase in mean RBC counts, PCV and Hb in the treated groups, especially in Groups A and C during the treatment period. However, during the post-treatment period, the remaining control pig's figures increased to match those of the remaining treated pigs, therefore it is possible that this observation was not an effect of prednisolone.

TABLE 10

PREDNISOLONE IN NORMAL PIGS

GROUP D - SALINE - TREATED CONTROLS GROUP A - LOW DOSE GROUP B - MEDIUM DOSE GROUP C - HIGH DOSE

DAYS FROM START OF DOSING	RBC - MEANS \pm 1 S.D. ($\times 10^{12}/l$)				PCV - MEANS \pm 1 S.D. (1/1)				Hb - MEANS \pm 1 S.D. (g/dl)			
	GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C
- 12	6.61 \pm 0.18	6.71 \pm 0.81	7.16 \pm 0.13	7.00 \pm 0.29	0.325 \pm 0.01	0.305 \pm 0.05	0.335 \pm 0.01	0.33 \pm 0	11.3 \pm 0.2	10.3 \pm 1.8	11.4 \pm 0.3	11.1 \pm 0.1
- 5	5.87 \pm 0.54	6.36 \pm 0.05	6.68 \pm 0.06	6.41 \pm 0.12	0.32 \pm 0	0.33 \pm 0.02	0.32 \pm 0.01	0.32 \pm 0	10.2 \pm 0.3	10.8 \pm 1.0	12.4 \pm 0.2	10.9 \pm 0.2
0	7.04 \pm 0.26	7.46 \pm 0.51	7.02 \pm 0.31	7.01 \pm 0.27	0.31 \pm 0.04	0.335 \pm 0.01	0.31 \pm 0.02	0.32 \pm 0	10.5 \pm 1.2	11.0 \pm 0.8	11.1 \pm 0.4	10.4 \pm 0.4
Start of Dosing												
7	7.07 \pm 0.16	7.61 \pm 0.21	7.52 \pm 0.21	7.24 \pm 0.21	0.33 \pm 0	0.325 \pm 0.02	0.325 \pm 0.04	0.32 \pm 0.01	10.0 \pm 1.3	10.8 \pm 0.8	11.3 \pm 0.8	10.6 \pm 0.1
14	7.18 \pm 0.29	7.44 \pm 0.11	7.85 \pm 0.09	7.44 \pm 0.02	0.295 \pm 0.05	0.325 \pm 0.02	0.335 \pm 0.02	0.33 \pm 0.01	10.1 \pm 1.3	10.8 \pm 0.6	11.4 \pm 0.4	10.9 \pm 0.4
21	6.83 \pm 0.18	7.31 \pm 0.23	7.88 \pm 0.12	7.31 \pm 0.54	0.31 \pm 0.01	0.315 \pm 0.02	0.345 \pm 0.01	0.33 \pm 0.01	9.8 \pm 0.3	10.7 \pm 0.4	11.7 \pm 0.5	10.8 \pm 0.1
28	7.20 \pm 0.68	7.77 \pm 0.11	7.66 \pm 0.26	7.55 \pm 0.10	0.315 \pm 0.02	0.35 \pm 0.01	0.34 \pm 0.01	0.34 \pm 0	10.2 \pm 1.0	11.4 \pm 0.6	11.3 \pm 0.6	11.1 \pm 0.4
35	7.00 \pm 0.04	8.17 \pm 0.86	7.61 \pm 0.37	7.78 \pm 0.30	0.325 \pm 0.01	0.355 \pm 0.02	0.355 \pm 0.01	0.35 \pm 0.01	10.6 \pm 0.4	11.9 \pm 0.6	11.6 \pm 0.6	11.4 \pm 0.1
End of Dosing												
42 x	7.20	7.48	8.07	7.58	0.32	0.35	0.35	0.36	11.2	11.4	12.3	11.3
49 x	7.12	7.94	7.87	7.87	0.32	0.32	0.36	0.32	11.0	11.0	11.6	11.2
56 x	7.23	7.26	7.47	7.56	0.33	0.32	0.32	0.34	11.4	10.6	10.9	11.4
63 x	7.13	7.23	7.90	7.52	0.34	0.33	0.35	0.335	12.1	11.2	11.6	10.7
70 x	7.57	7.53	7.81	7.48	0.36	0.36	0.35	0.32	12.4	11.5	11.9	11.0

x one pig in each group

The derived red cell indices showed no changes which could be attributed to the treatment.

Table 11 shows mean thrombocyte counts \pm 1 S.D. Counts in the treated groups on day 28 were much lower than those of the control group, but there was a rise in these mean counts by day 35, and none of the treated pigs had a dangerously low count at any time during the experiment.

Serum Electrolytes

The results are shown in Tables 25 to 32 in Appendix I. Levels fluctuated but there were no differences between groups which could be attributed to the treatment, and in particular, there was no evidence of sodium retention or of potassium or calcium depletion in the treated groups.

Serum Proteins

Although no pretreatment serum samples were taken, it appears from Table 12 that mean serum albumin levels increased in all groups during treatment but that the increase was greater in Groups A, B and C than in the control group. Serum globulins were obtained by subtraction of serum albumin from total protein values (See Appendix I Tables 33, 34). In Group D, mean serum globulins increased during treatment, whereas in all treated groups there was a marked decrease. The lowest levels were recorded on day 28, before the end of treatment.

TABLE 11

PREDNISOLONE IN NORMAL PIGS - MEAN THROMBOCYTE COUNTS ± 1 S.D. $\times 10^9/L$

DAYS FROM START OF DOSING	GROUP D (CONTROLS)	GROUP A (LOW DOSE)	GROUP B (MEDIUM DOSE)	GROUP C (HIGH DOSE)
- 12	300 \pm 245	348 \pm 37	284 \pm 13	261 \pm 13
- 5	610 \pm 181	512 \pm 3	566 \pm 217	538 \pm 5
0	638 \pm 382	530 \pm 22	555 \pm 9	520 \pm 56
Start of Dosing				
7	580 \pm 243	683 \pm 182	637 \pm 32	553 \pm 54
14	438 \pm 100	536 \pm 192	531 \pm 18	477 \pm 16
21	517 \pm 235	590 \pm 52	491 \pm 53	478 \pm 110
28	419 \pm 137	244 \pm 28	196 \pm 54	263 \pm 47
35	502 \pm 94	346 \pm 48	468 \pm 28	387 \pm 40
End of Dosing				
42 +	297	391	470	364
49 +	315	479	365	309
56 +	321	450	459	380
63 +	261	388	405	312
70 +	252	390	383	251

+
1 pig only in each group

Table 12

Why are
globulin
levels so
much lower
in control
group after
Rx stopped?

Post-Mortem Findings - Gross PathologyControl Pigs

2 - killed at end of treatment.

This was a rather small, thin pig, but normal apart from about 50 ml clear fluid in the abdominal cavity.

4 - killed 5 weeks later.

This was a normal pig, except for the caecum in which the mucosa was slightly thickened and nodular.

Low-dose pigs

1 - killed at end of treatment.

This was an anatomically normal carcase, with no fluid in chest or abdomen, and only a slight excess of fluid in the pericardial sac.

The thymus was composed of rather small, brown, firm nodules.

The liver was slightly pale.

The bone marrow of the femur was red in the cancellous tissue at either end with white soft fat in the shaft.

6 - killed 5 weeks later.

This was also a normal pig, showing slight fluid in the pericardial sac and abdominal cavity only.

Medium-dose pigs

5 - killed at end of treatment.

This was a normal carcase. The thymus was quite large with pale buff-coloured lobules.

8 - killed 5 weeks later.

This was also a normal pig. There was a slight amount of clear fluid in the pericardial sac.

Peyer's patches in the ileum were very distinct.

High-dose pigs

7 - killed at the end of treatment.

This was a small, thin pig, having similar amounts of fat compared with the others, but not as muscular. The carcass showed no abnormalities.

There was no fluid in chest or abdomen.

The thymus was small with brown, firm nodules.

The bone marrow of the shaft of the femur was dull pink throughout its length, but it floated in formalin.

3 - killed 5 weeks later.

This was a normal carcass, showing a slight amount of clear fluid in pericardium, chest and abdomen.

Histological Examination

Microscopic examination of the tissues from these pigs, and from the lymphosarcoma pigs in the following experiment, was carried out by Mr. K.W. Head, Department of Veterinary Pathology, Royal (Dick) School of Veterinary Studies. The results described in this chapter are summarised from his reports.

Tissues from pigs killed at the end of treatment

The thymus in each of the four pigs, including the control, was involuted. Of the treated pigs, the involution was most marked in the high-dose animal, and

showed as a reduction in lobule size, the lobules being separated by fat and connective tissue. Both cortex and medulla were reduced, cortex more than medulla. However the density of the cell population of both cortex and medulla was normal.

In the spleens, there was some variation in size and number of nodules and degree of activity of the white pulp but it did not correlate with dose regime, time of death or weight of spleen.

There were no differences in the total width of the adrenal cortex between animals.

In all the pigs, the Peyer's patches showed reaction centres with mitotic figures, except in the high-dose pig in which the nodules were smallest and the cells in the centre were inactive.

The lymph-nodes in the high-dose pig, 7, were distinctive in several respects. In contrast to the control and other treated pigs, a) the submandibular node showed many "blast" cells with numerous mitotic figures, both in the reaction centres and in some of the paracortical regions where there was a reduction in small lymphocytes, b) the prescapular node had almost no lymphocytes in the cortex, c) the medulla of the mesenteric node was reduced in size and the paracortical zone was more extensive than usual, and d) the external iliac node showed more foci of megakaryocytes and normoblasts in the medulla.

Lung, liver, kidney and stomach were normal and similar in all the pigs. There was no evidence of osteoporosis.

The bone marrow samples varied in cellularity but they showed only differences that could be attributed to age and growth rate.

Tissues from pigs killed 5 weeks after stopping treatment

Cryostat sections of the caecum of control pig 4 showed that the mucosal thickening was not due to adenomatosis. Smears showed numerous spirochaetes.

The thymus in each of the four pigs was histologically normal.

The external iliac lymph nodes of all the pigs showed more extramedullary haemopoiesis than in any of those killed earlier. In addition, the ratio of cortex to medulla was reversed from 1:3 to 3:1, this being due to an increase in size of both the reaction centres and the paracortical zone.

Lymph node and Organ Weights

See Appendix I for absolute weights in grams. Table 13 shows the effects of prednisolone by calculation of the relative weights in centigrams/kilogram body weight of lymph nodes and organs. In this way, results from pigs of differing body weights may be compared. At the end of the treatment period, there was a dose-related reduction in relative weight of the

TABLE 13

RELATIVE WEIGHTS OF LYMPH NODES AND ORGANS (cg/kg body weight)

Prednislone - Normal Pigs

DOSE	KILLED AT END OF TREATMENT				KILLED AT END OF EXPERIMENT			
	CONTROL	2mg/kg	4mg/kg	6mg/kg	CONTROL	2mg/kg	4mg/kg	6mg/kg
Pig No	2	1	5	7	4	6	8	3
Lymph Nodes :								
Mesenteric & Colonic	523	341	324	374	408	325	250	275
Gastric & Splenic	33	28	25	24	31	34	32	32
Bronchial Group	11	13	9	8	10	8	7	11
Total Splanchnic	567	382	358	406	449	367	289	318
Head Group	30	27	30	24	24	28	21	21
Iliac Group	8	10	6	7	8	10	8	6
Prescapular	3	2	7	4	3	5	3	5
Preauricular	4	3	3	2	2	3	2	3
Inguinal	15	12	13	9	12	10	10	10
Popliteal	2	2	1	0.4	NF	1	1	1
Total Superficial	62	56	60	46	49	57	45	46
Thymus	91	69	38	26	133	203	185	210
Spleen	227	185	121	212	122	125	87	138
Liver	3500	3741	3862	3234	3306	3325	2731	3075
Kidney	250	259	241	200	167	175	183	188
Adrenal	5	7	6	6	4	6	4	6

thymuses of the treated pigs. When compared with the control, in which the thymus was also more involuted than would be expected in a normal pig of this age, the reduction amounted to 24, 58 and 71% in the low, medium and high dose pigs respectively.

In the pigs killed 5 weeks after treatment was discontinued, the relative thymus weights represented 156, 142 and 165% respectively of the corresponding control value.

There was also a reduction in relative weights of the lymph nodes of the treated pigs killed at the end of treatment. This was not dose-related, but averaged 23% in the splanchnic nodes and 13% in the superficial nodes.

In the pigs killed at the end of the experiment, the relative weights of the superficial nodes were similar to those of the control, but the splanchnic nodes still averaged only 72% of the relative weights of the control's splanchnic nodes.

DISCUSSION

The effects of corticosteroids in man have already been described (see Introduction to Chapter I).

Before discussing the results of this experiment, it was necessary to consider the effects of corticosteroid administration in the other domestic species.

There are many reports in the veterinary literature concerning the uses of corticosteroids in animals. These uses were reviewed by Uvarov (1959), Sodikoff (1966) and Austin (1971).

Despite the many reports of therapeutic effects of corticosteroids in a wide variety of animal diseases, there are very few reports which consider their pharmacological and toxicological effects in any of the domestic species, and those that do are often descriptions of the immediate effects of a single dose or short-term treatment.

As early as 1944, Reinhardt, Aron and Li showed that injection of ACTH produced a lymphopenia and neutrophilia in normal rats and dogs. Demanet, de Meutter and Gepts (1958) investigated the metabolic effects of long-term cortisone treatment in dogs. Treated dogs became obese on an ad lib diet rich in carbohydrate, showed increases in α_2 globulin component of serum protein, alterations in carbohydrate and fat metabolism, and adrenal atrophy but no change in serum electrolytes.

Fielder, Hoff, Thomas, Tolksdorf, Perlman and Cronin (1959) studied the toxicity of prednisolone, methylprednisolone and triamcinolone in dogs fed a standard ration. They were treated orally for 6 weeks with 2.5 and 5 mg/kg daily. Triamcinolone caused death with emaciation, gastro-intestinal haemorrhage, pneumonia and pleural effusion, pyogenic infection, and possibly bone marrow toxicity. Weight loss was pronounced with triamcinolone, and occurred with methylprednisolone at the higher dose rate, but not with prednisolone. Diuresis, and falls in PCV and Hb also occurred with triamcinolone.

All treated dogs showed increased urinary excretion of sodium.

The effects of ACTH, flumethasone and dexamethasone in horses were studied by Osbaldiston and Johns on (1972). Leucocytosis, lymphopenia and neutrophilia were recorded for 17 hours after one injection. There was apparently an eosinophilia but no effect on platelets, RBC counts or monocyte counts. Targowski (1975) described similar effects in ponies given a single intravenous dose of prednisolone, except that he found depression of eosinophil counts. He also tested dermal response to an antigen to which the ponies were sensitive, and found that the delayed hypersensitivity reaction was suppressed by prednisolone.

Goetsch, McDonald and Odell (1959) investigated the effects of one injection of each of four synthetic corticosteroids in the cow. All the preparations caused neutrophilia and mild eosinopenia, and raised blood glucose levels, but lymphocytes and plasma sodium and potassium were unaffected.

The immediate effects of ACTH and adrenal cortical extract on the blood picture of the pig were investigated by Luke (1953). He found that they produced an increase in absolute neutrophil counts, with a peak between 4 and 8 hours after injection, and a lymphopenia 2 hours after injection. Patt and Eberhart (1977) used ACTH in newborn piglets and found no statistically significant effects on the WBC picture. Wulf (1969) investigated the immediate effects of prednisolone and dexamethasone in pigs. The dose of prednisolone used was 1.5 mg/kg, given intramuscularly and the pigs were blood-sampled at 30 minutes, and at 1, 2, 3, 4, 7 and 9 hours. A slight fall in mean lymphocyte counts and a marked neutrophilia were recorded, particularly between 3 hours and 7 hours after dosing. Seidel, Müller, Kolb and Pohle (1967) administered prednisolone to pigs for 14 days and showed that similar effects occurred, and were maintained throughout the treatment period. There was a fall in eosinophil counts after administration of ACTH, which was administered twice during prednisolone treatment and once after it was stopped. There was a rise in blood glucose, but no effects on serum

electrolytes.

Attempts to find other literature reports of long term administration of corticosteroids in normal pigs have been unsuccessful.

The mild clinical effects of prednisolone treatment, in the experiment described here, particularly in the high-dose group which were given 6 mg/kg, are fairly typical of the effects recorded in man. The marked "pot-bellied" appearance was not due to ascites or any disease of the abdominal organs, and disappeared when the animals were laid on their backs, therefore it must be assumed that it was due to abdominal muscle weakness. Depression of weight gain in pigs, given the same and higher dose-levels of prednisolone for 19 days, was noted by Zamora, Kowalczyk, Hoekstra, Grummer and Will (1975).

The neutrophilia, expected from the many reports of this in the literature in man and animals was not observed in any of the treated pigs, even in three pigs which had normal neutrophil counts at the start of the experiment. Videbaek (1969) stated that the neutrophilic response to corticosteroid administration has several components. Firstly there is a transitory release of neutrophils from the "marginal pool" i.e. those which "stick" to blood vessel walls and are not normally counted in WBC counts. These are thought to be the first to go to sites of tissue inflammation. Secondly, there is a reduction in the

migration of granulocytes from the blood stream. Thirdly, if corticosteroid administration is continued there is thought to be an overproduction of neutrophils, with premature release, through an apparently direct influence on the bone marrow.

In man, (Myles and Daly, 1974) and in pigs (Seidel et al., 1967) it has been shown that the neutrophilia persists while treatment lasts, though in neither case was the route of administration specified, therefore timing of the weekly blood-sampling was unlikely to be the reason why it was not detected. The pigs were not ill and there was no shift to the right, therefore there was no question of the bone-marrow being unable to respond. Two of the pigs used by Wulf (1969) had neutrophilia prior to intramuscular prednisolone treatment, which became even more pronounced after treatment.

The dose-related fall in lymphocyte counts did correspond with corticosteroid effects recorded in man and other animals. However, the eosinopenia and monocytopenia expected from the literature reports did not occur. It is possible that different results might have been obtained if a special staining and counting method for eosinophils has been used.

The observed depression of serum globulin and increase in serum albumin levels associated with treatment are similar to the effects described in arthritic swine treated with cortisone by Shetlar, Shetlar, Payne, Neher and Swenson (1958). These effects have

also been described in man in relation to corticosteroid therapy of chronic diseases. Mistilis and Lam (1972) reported a decrease in serum globulins and an increase in serum albumin in patients with chronic active hepatitis treated with prednisolone. They suggested that the relationship between serum albumin and globulin levels may be reciprocal.

The extent and rate of development of the effects on the thymus, lymphnodes and blood lymphocytes of the treated animals suggests that the pig belongs to the corticosteroid-resistant group of species, which includes man, the ferret and the guinea-pig (Claman, 1972). In the corticosteroid-sensitive mouse, rat, hamster and rabbit, the lytic effects are much more severe and rapid at comparable dosage. The reduction in size of the thymus during treatment, and its recovery with "overshoot" after withdrawal of treatment, is a phenomenon which has been described in non-lymphomatous children treated with prednisone and triamcinolone, (Caffey and Silbey 1960).

The corticosteroid suppression of cell-mediated immunity recorded above also places the pig within the steroid-resistant group of species, since it required large doses and prolonged treatment. In man, for example, prednisone given orally in 40 mg/day doses had to be administered on average for 13.6 days to negate the effects of the tuberculin test (Bovornkitti, Kangsadai, Sathirapat and Oonsombatti, 1960).

Zamora et al., (1975) carried out a similar type of

experiment in pigs to investigate the effects of intramuscular prednisone administration on gastric secretion and development of stomach lesions. All their treated animals showed pathological changes in the glandular region of the stomach, such as interstitial haemorrhage, catarrhal and haemorrhagic gastritis and erosions. Since prednisone is converted to prednisolone in the body, and similar dose levels were given to pigs of comparable age, the absence of gastric lesions in our treated pigs is difficult to explain. It should be noted, however, that two of the five control animals used by Zamora et al., (1975) also showed gastric lesions, therefore other factors may have been operating. Gastric ulceration is common in pigs, although it usually involves the non-glandular oesophageal region of the stomach, (Kowalczyk, 1975). Many factors are suspected to be involved, the most important of which is food particle size. However, Zamora et al., (1975) did show that 10 mg/kg for 8 days temporarily increased the volume and acidity of gastric secretion. They also discussed reported evidence in pigs and other species which suggests that fasting may encourage the development of gastric lesions. Their pigs were fed only once daily whereas our pigs were fed twice daily, and this may have helped to protect the gastric mucosa of our pigs in some way.

From this experiment, it is evident that the reactions of normal pigs to prednisolone show some promising

similarities to the effects recorded in man. The results were of use in planning the therapy trial, and since a dose level of 6 mg/kg was apparently capable of producing a degree of toxicity, 4 mg/kg was chosen as the dose level for the lymphosarcomatous pigs.

PREDNISOLONE THERAPYExperiment AMATERIALS AND METHODSAnimals and Pre-treatment Procedures

Four female pigs were used for the therapy trial. Two were 6 months old and two were 5 months old at the start of the experiment, the latter pair being litter-mates. Lymphosarcoma had been diagnosed when they were between 8 and 10 weeks of age. The four pigs were housed together in the same pen. In addition to the normal feed (see General Methods), a vitamin-mineral supplement ("Nutrequin", Berk) was given throughout, as the pigs were in poor condition before the experiment started and supportive therapy was considered necessary.

Weekly weighing and blood-sampling commenced 4 weeks before the start of treatment. As in the trial using normal pigs, EDTA samples were taken for haematology, and clotted samples for measurement of serum sodium, potassium, chloride, calcium, magnesium, phosphate, total protein and albumin. A detailed clinical examination was carried out weekly from 2 weeks before treatment started. Rectal temperatures were recorded daily and lymph node and abdominal measurements were taken weekly as described in "General Methods". Sensitisation with DNFB was carried out at -3 weeks and skin-testing at -11 days.

Treatment

As in the trial using the normal pigs, the drug used was prednisolone sodium phosphate, 16 mg/ml ("Codelsol", MSD). However, during the 3rd week of treatment, "Codelsol" was unavailable and it was necessary to use other preparations ("Vecortenol" - prednisolone pivalate 10 mg/ml, Ciba-Geigy, and "Nisolone", 10 mg/ml prednisolone, Bimeda).

The prednisolone dose level used for all four pigs was 4 mg/kg, administered intramuscularly at the same time each day for 5 days/week, using injection sites in rotation, as in the normal pig trial. As before, doses were adjusted for weight gain.

Prophylactic oral antibacterial therapy was given during the period of prednisolone dosing. The preparation used was framycetin sulphate ("Framomycin Feed Additive", C. Vet.).

During treatment the pigs were examined and rectal temperatures recorded daily at the time of each injection. Weighing, blood-sampling and lymph node measurements were carried out weekly as before. Skin-testing was performed after 4.5 weeks of therapy. After 5.5 weeks, two pigs, one of each age pair, were killed by pentobarbitone overdose, and autopsied. Treatment of the survivors was tailed off during the following three days. These animals were examined and blood-sampled weekly thereafter, but no further

treatment was administered and temperature recording was stopped. Skin-tests were carried out 4 weeks after stopping prednisolone. After 5 weeks, the remaining pigs were also destroyed and autopsied.

At the time when this experiment was in progress, a non-lymphosarcoma/^{tous}female littermate of the younger pair of pigs developed a right deviation of the nasal bones. It had been used as a control for the measurements of skin hypersensitivity to DNFB and two days after one of its siblings had been destroyed at the end of treatment, this animal was also killed. The deformity did not have an infectious cause and was considered to be congenital. This was the only abnormality in the carcass, and so it provided a useful age and sex-matched normal control for the autopsy lymph-node and organ weights. Data from 17 untreated lymphosarcoma cases which had died or been killed at comparable ages to the treated pigs, were also used in the assessment of the autopsy results.

RESULTS

Clinical Examinations (see Clinical Records, Appendix I).

Before treatment, all four pigs ate fairly well but were less active and easier to handle than normal pigs of similar age. Weight gains were poor, and one pig had previously had persistent diarrhoea. Within a few days of starting prednisolone therapy, there was a marked improvement in appetite and vigour. The pigs became more interested, active and more difficult to handle. As in the previous experiment, the pigs drank more frequently and appeared to pass more urine, though this could not be measured. The "pot-bellied" appearance, typical of the lymphosarcomatous pig, did not disappear, and in fact became more marked. ^{Plates 2 and 3.} However abdominal measurements taken with the pigs lying on their backs remained fairly steady in three animals, and actually decreased progressively from 88 cm to 80 cm in the fourth (3637) during treatment. There was no clinical evidence of ascites, and little or no fluid could be withdrawn by paracentesis abdominis. All pigs had normal faeces throughout the experiment.

Two pigs developed cellulitis from abrasions and bite wounds during treatment. Procaine penicillin ('Mylipen', Glaxo Vet) was administered intramuscularly for 3 days and recovery was rapid. The rectal temperatures of these pigs were fairly high when the infection was noticed, but temperatures as high were

TABLE 14

Prednisolone TherapyLymph node measurements - mean products of lengths and breadths of inguinal and precrural nodes (mm²).

WEEK	PIG NO. 3630		PIG NO. 3737		PIG NO. 3652		PIG NO. 3656	
	Inguinal	Precurral	Inguinal	Precurral	Inguinal	Precurral	Inguinal	Precurral
-1	1662	1531	2340	1157	2067	1218	1593	915
0	1892	930	1863	897	1716	850	1439	906
START OF DOSING								
1	1416	640	1736	585	1428	524	1152	293
2	1105	562	989	476	1020	428	890	300
3	809	342	918	369	915	356	782	294
4	1144	Very small	863	317	885	Very small	Very small	Very small
5*	NOT MEASURED		765	Very small	775	Very small		NOT MEASURED
END OF DOSING								
6	DESTROYED		1150	Very small	954	Very small		DESTROYED
7			1050	Very small	1050	Very small		
8			1272	Very small	1986	Very small		
9			1458	689	2218	736		
10			1967	865	2237	1080		

* During Week 5 the nodes were too small and soft to make accurate measurements.

recorded at other times with no evidence of other signs of fever. (See Clinical Records, Appendix I).

During the 4th week of treatment, pig 3630 was noticed to be trembling. It was depressed, though not anorexic. Its rectal temperature was rather low at 38.0°C. Prednisolone treatment was continued, but it was separated from the other animals, to prevent bullying, and given a heat lamp. The trembling lasted for 2 days. When blood sampled the previous week, its serum potassium level had been low (see serum electrolyte results p. 155). Since the trembling may have been due to potassium loss, 1g potassium chloride was administered by mouth, mixed with treacle, once daily for 2 days until the trembling stopped.

After prednisolone therapy was withdrawn, the two surviving animals remained active with good appetites, but after 5 weeks, they were again pale and anaemic-looking. There was no sign during the post-treatment period, of adrenal insufficiency.

During prednisolone treatment, there was a dramatic shrinkage of superficial lymph nodes (see Table 14). By the 3rd week of treatment, all four pigs showed a reduction in the mean products of lengths and breadths of inguinal nodes of at least 45%, and pre-crural nodes of at least 58%. By the 5th week of treatment, the superficial nodes were so small and soft that they could not be measured. During the post-

treatment period, the nodes in the remaining pigs gradually increased in size again, until they were as large as they had been before treatment started.

See plates 4 and 5.

TABLE 15

Prednisolone therapy - body weight gains (kg)

Pig No.	Pretreatment (4 weeks)	During Treatment (5.5 weeks)	After treatment (5 weeks)
3630	- 0.5	2	Dead
3656	4	6	Dead
3637	3	0.5	9
3652	2.5	2.5	6

Weight Gains

During the observation period before the start of the experiment, weight gains were poor and one pig actually lost weight (See Table 15). During treatment, all four pigs gained weight but there was little difference from the weight gain during the pretreatment period. After the prednisolone was withdrawn, however, both the remaining animals increased their weight gain, producing similar results to those obtained in the previous experiment (See Page 129) with normal pigs.

DNFB Skin-Test Results

The reaction scores in Table 16 show that skin hypersensitivity to 10% DNFB was minimal or absent in all four lymphosarcomatous pigs before therapy began.

TABLE 16

Prednisolone TherapyDNFB Skin Test Results

Pig No.	At Sensitisation	Pre-treatment	Last Week of Treatment	1 Month Post-treatment
3630	3	0	2	Dead
3656	1	0	2	Dead
3637	0	0	2	2
3652	1	1	2	2
3654 NORMAL	1	4	Not tested	Dead

During and after therapy, a response of score 2 (erythema and swelling) was apparent. The untreated littermate of two of the lympho/^{sarcomatous} pigs was only tested on one occasion after sensitisation. However, it was useful to compare its much more severe reaction (large ulcerations) with the pretreatment scores of the treated pigs. It would appear therefore that treatment with prednisolone improved the cell-mediated response of the lympho/^{sarcomatous} pigs, the opposite effect to that observed in the previous experiment (see page 129) but that the response was still poorer than that which would be expected in normal untreated pigs.

Haematology

Before the start of prednisolone therapy, all four lympho/^{sarcomatous} pigs had high WBC counts and variable numbers of abnormal mononuclear cells were present in blood smears. In pigs 3630 and 3637 these cells were particularly numerous, and, in the latter animal, accounted for around 90% of the WBCs in differential counts (See Appendix I).

The effects of prednisolone therapy on mean absolute mononuclear and neutrophil counts are shown in Figures 2 and 3. There was a dramatic fall in the absolute mononuclear cell counts. The lowest mean value obtained was $4.1 \pm 0.5 \times 10^9/l$ during the 5th week of treatment when no recognisable tumour cells were observed in blood smears. During the 5 weeks

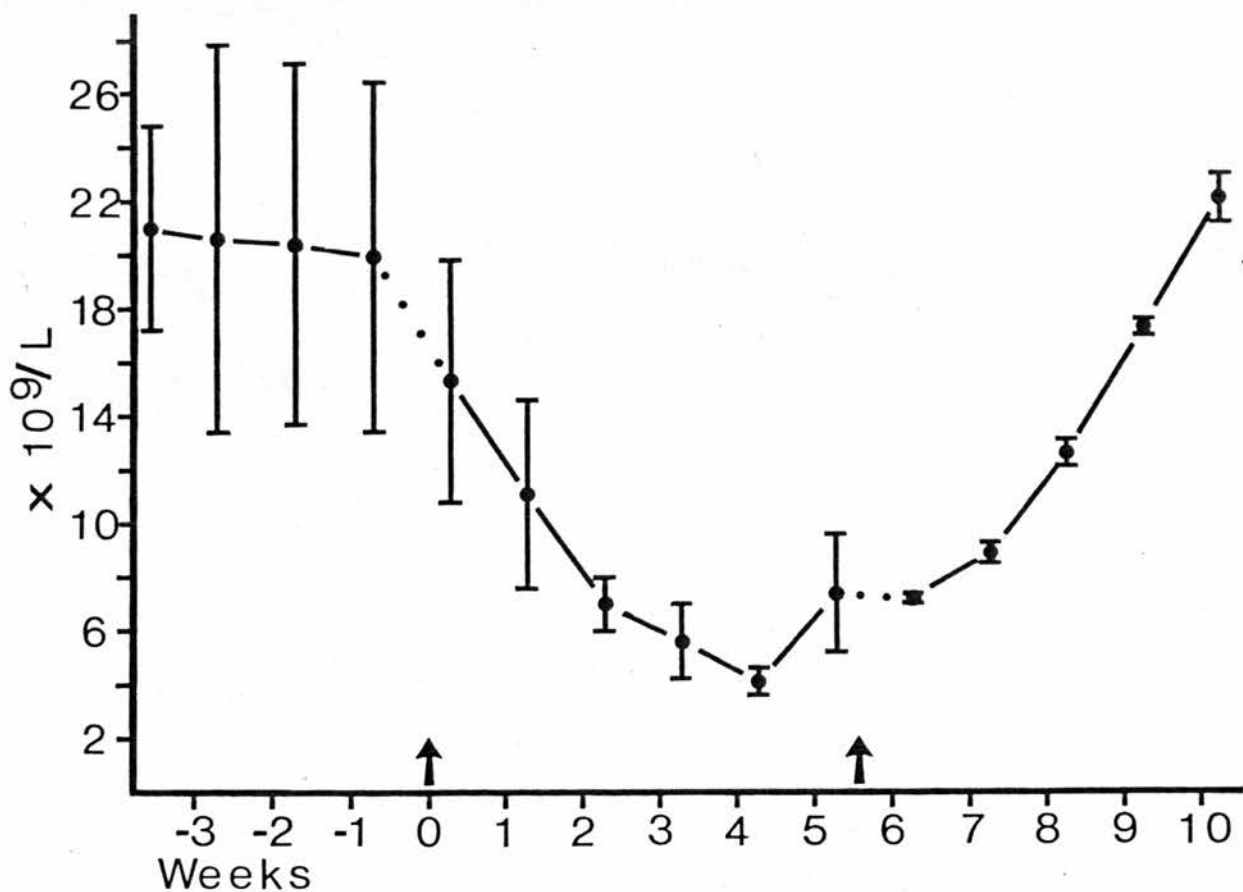
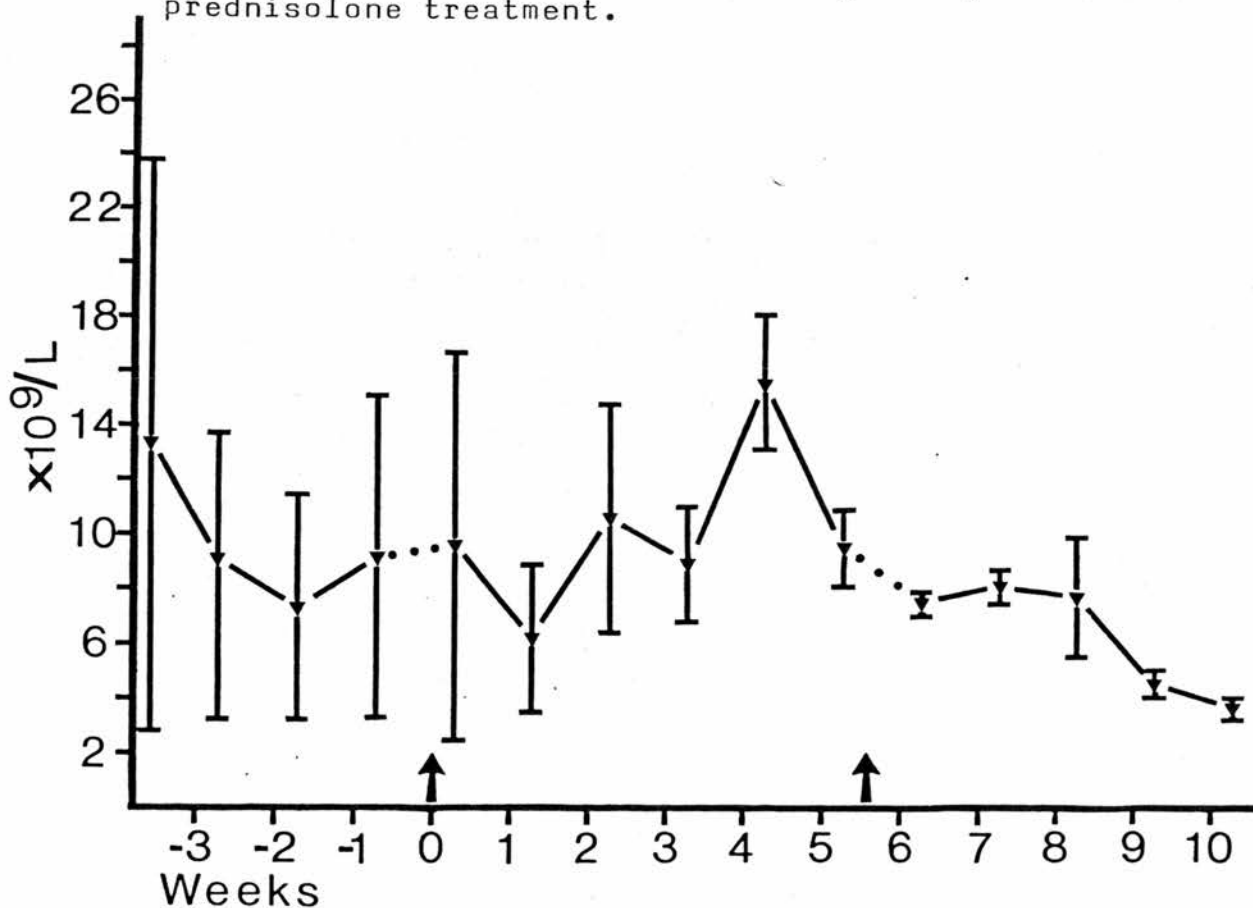


FIGURE 2 (above)
Absolute mononuclear cell counts (means \pm 1 S.D.) and

FIGURE 3 (below)
Absolute neutrophil counts (means \pm 1 S.D.) of lympho-
sarcoma cases. Arrows indicate beginning and end of
prednisolone treatment.



after treatment was withdrawn the mononuclear counts of the two surviving pigs rose progressively to approximately pretreatment levels, and abnormal cells reappeared in blood smears in large numbers.

Absolute neutrophil counts fluctuated widely in individual pigs, but the mean counts fell slightly during the 2nd week of treatment, rising to a peak of $15.6 \pm 2.5 \times 10^9/l$ during the 5th week and then falling again at the end of treatment.

Eosinophil counts remained at a low level throughout the experiment and so any effect is difficult to assess. However, they were observed in blood smears as frequently during prednisolone therapy as before or after. This was also true of basophils.

Mean RBC counts, Hb and PCV tended to fall during the pretreatment period, but rose rapidly from the commencement of treatment until about the 3rd week when they had almost reached normal levels. This improvement was maintained in the surviving pigs until the 2nd week after treatment ceased, after which there was a rapid fall in the red cell parameter values. By the end of the experiment, both pigs were once more anaemic. These changes are shown in Figures 4, 5 and 6.

The only discernible treatment-related effects on the derived red cell indices were in the surviving pigs during the post-treatment period, when the MCV fell immediately after the end of treatment and then rose from a mean of 51.0 ± 1.6 fl to 59.1 ± 5.1 fl.

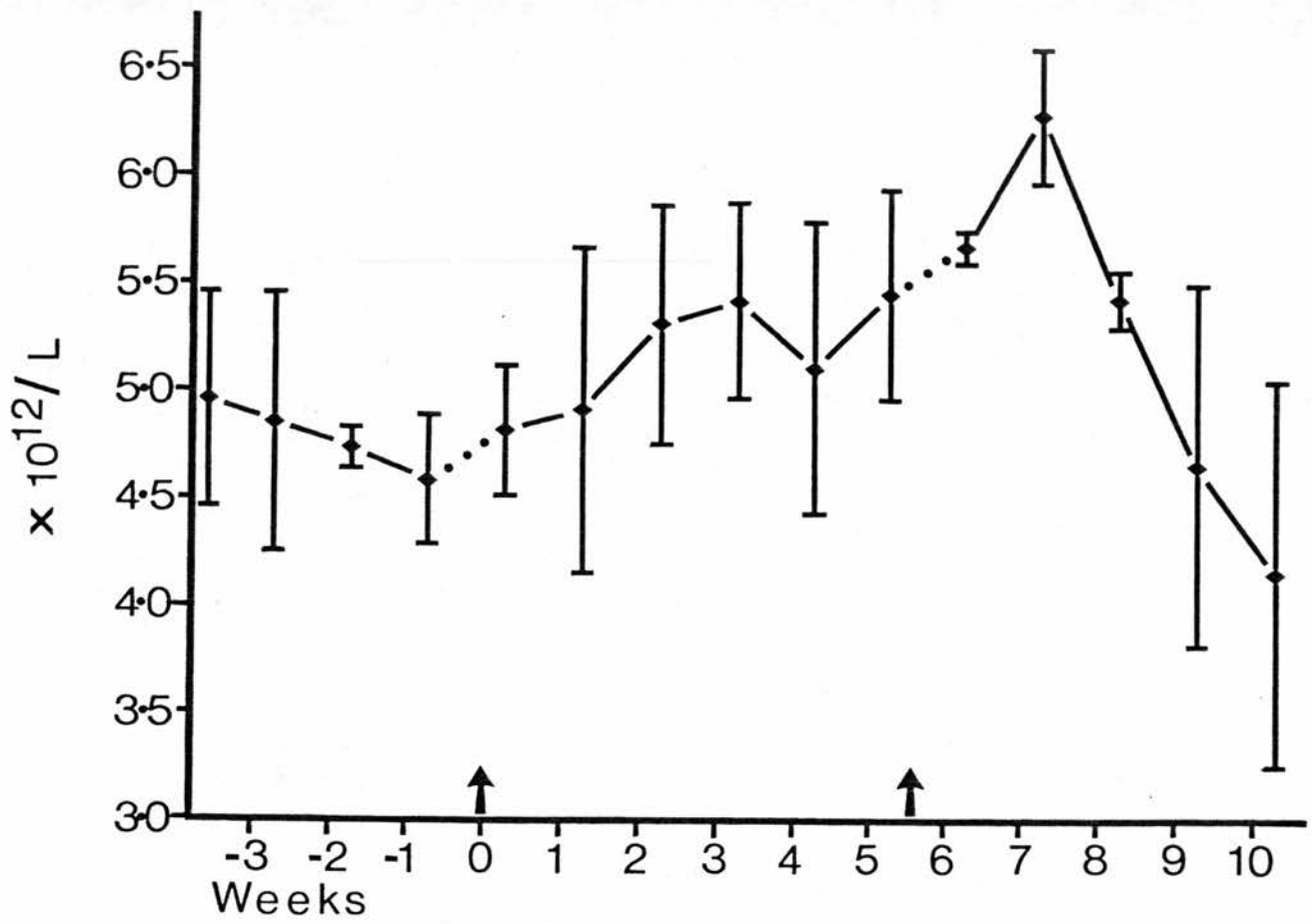
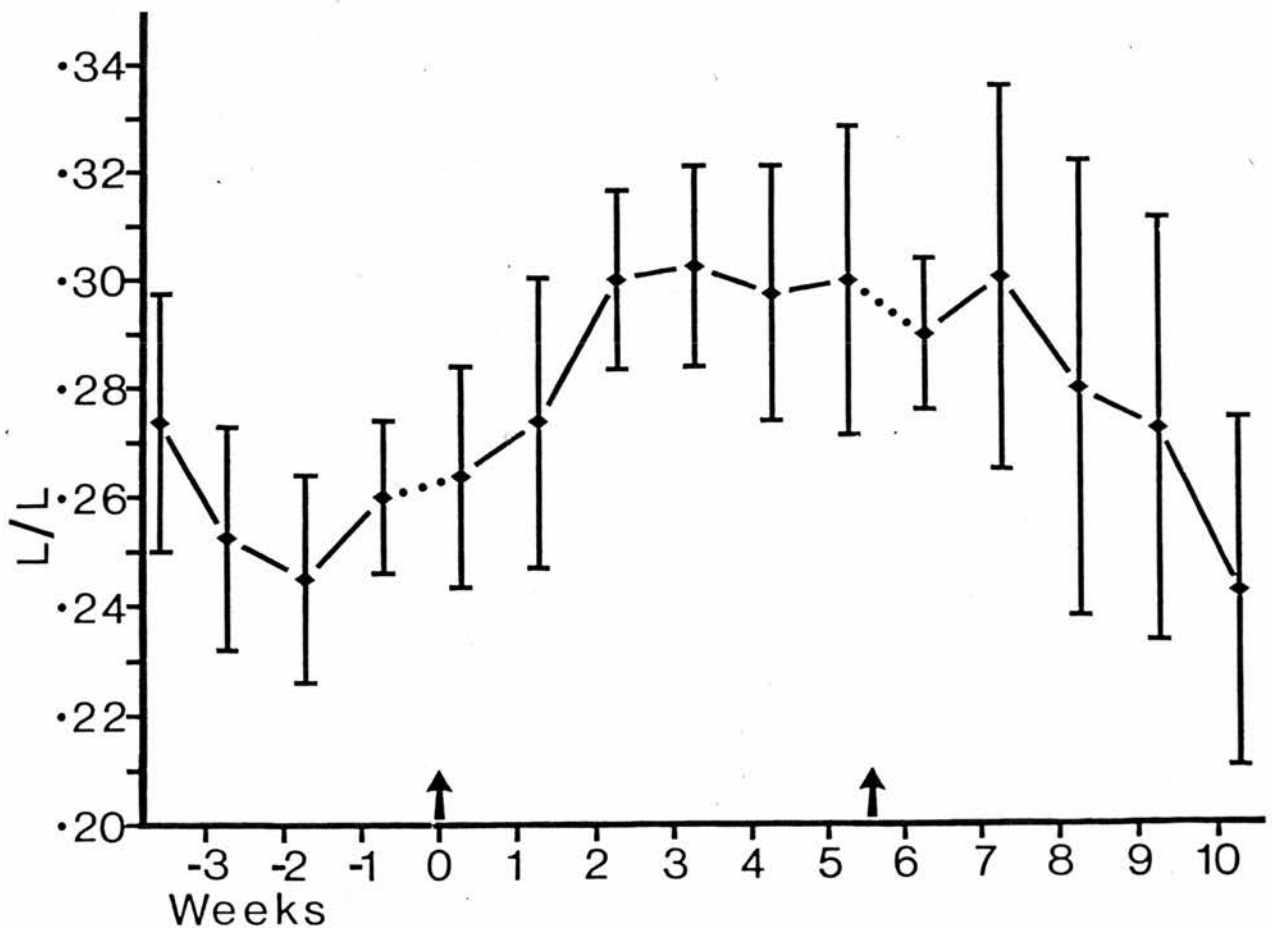


FIGURE 4 (above)
RBC counts (means \pm 1 S.D.) and

FIGURE 5 (below)
PCV (means \pm 1 S.D.) of lymphosarcoma cases. Arrows indicate beginning and end of prednisolone treatment.



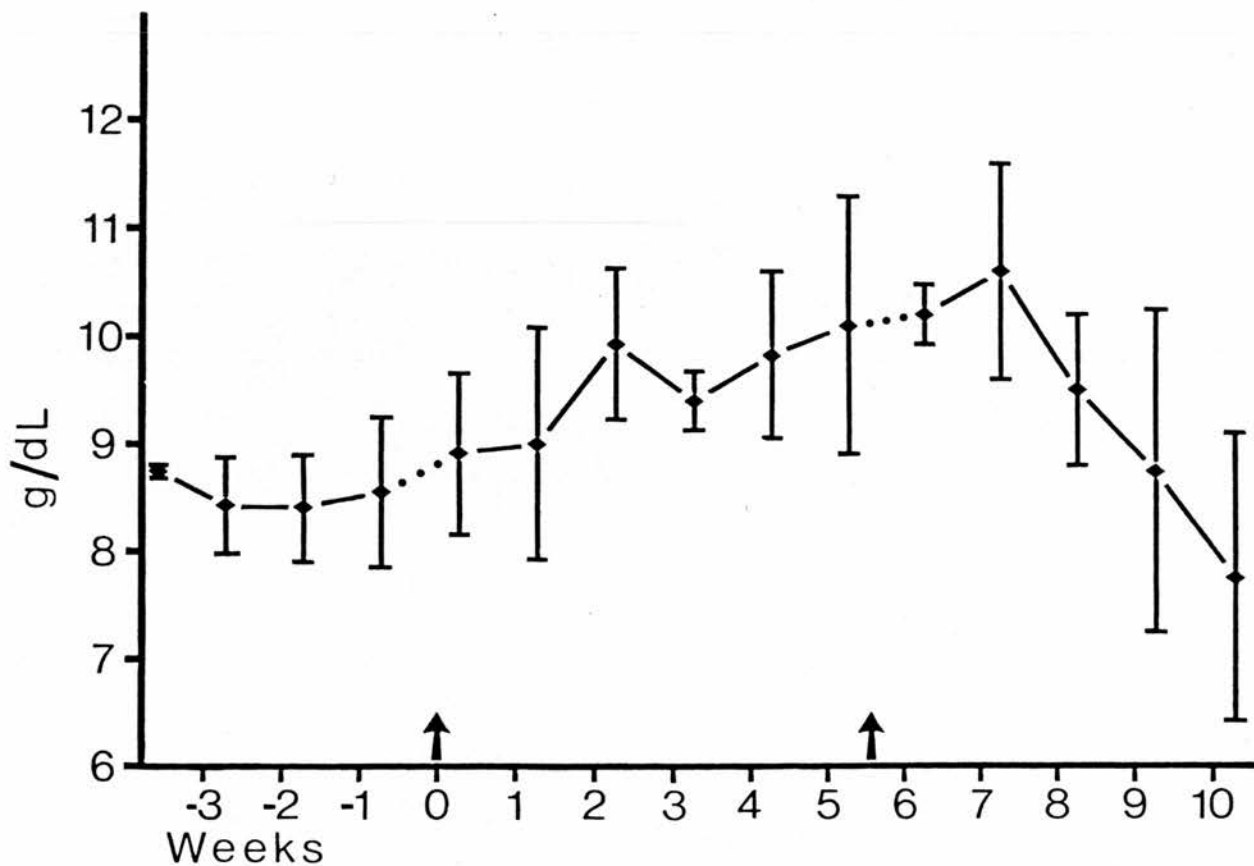
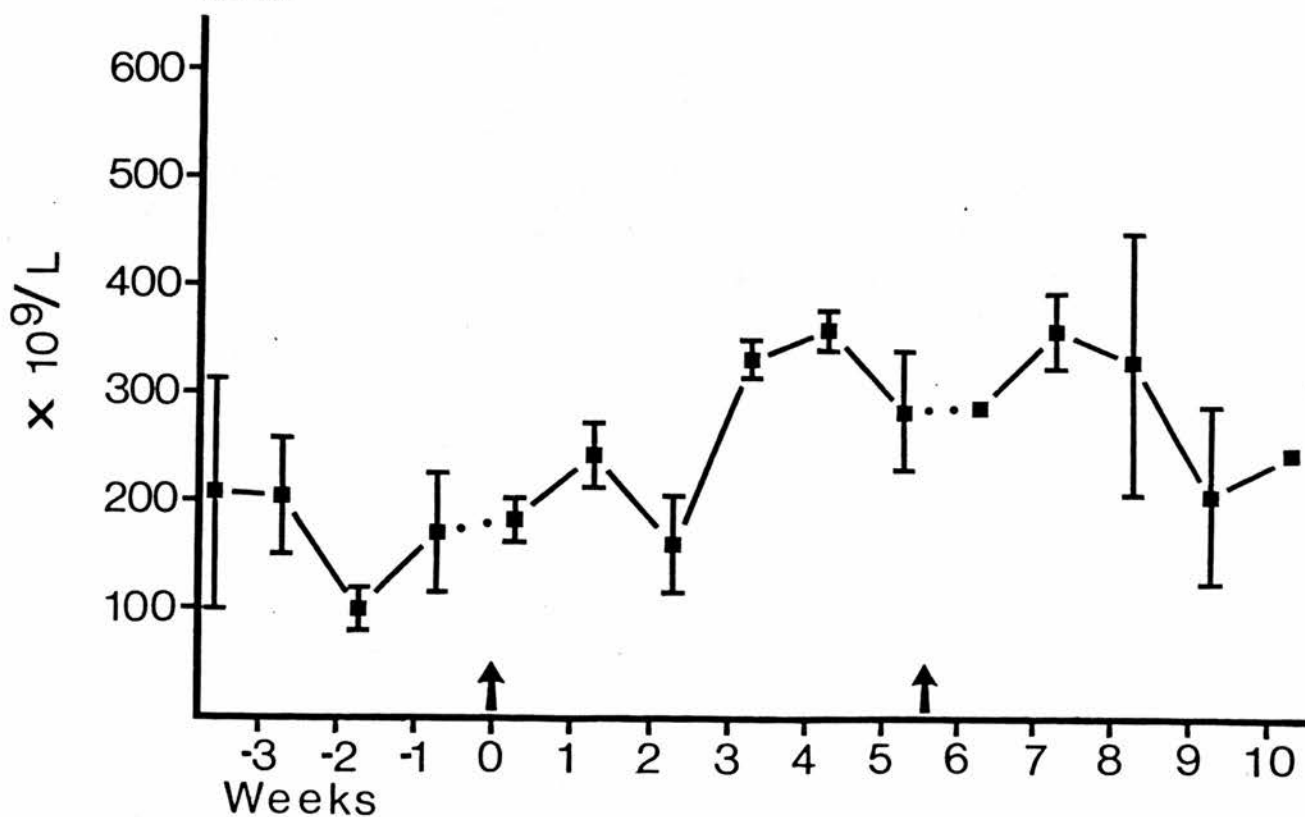


FIGURE 6 (above)

Hb (means \pm 1 S.D.) and

FIGURE 7 (below)

Thrombocytes (means \pm 1 S.D.) of lymphosarcoma cases. Arrows indicate beginning and end of prednisolone treatment.



Two weeks before treatment started, all four pigs had low thrombocyte counts (mean $99 \pm 20 \times 10^9/l$). (See Figure 7 and Appendix I.) By the 5th week of treatment, the mean had risen to $359 \pm 19 \times 10^9/l$. (See Figure 7.) By the end of the experiment, the mean count had fallen again in the two remaining animals but neither were thrombocytopenic at this stage.

Serum Electrolytes (See Table 17 and Appendix I).

The mean serum magnesium concentration, which had remained fairly steady before treatment started, showed upward fluctuations during treatment (See Figure 8) but was still within the normal range for the pigs in this piggery (0.8 to 1.25 mmol/l). After treatment ceased, in the two surviving pigs it fell immediately.

The mean serum potassium concentration changed little throughout the experiment and was within the normal range (4.0 to 5.4 mmol/l). However, pig 3630 had a potassium level of 3.5 mmol/l during the 3rd week of treatment, just before showing clinical signs which could have been due to potassium deficiency. The following week, after potassium had been administered by mouth, its serum potassium level had risen to 5.2 mmol/l.

Mean serum sodium, chloride, calcium and inorganic phosphate showed fluctuations but revealed no changes which could be attributed to the treatment. The range in serum sodium levels recorded during the experiment

TABLE 17

SERUM BIOCHEMISTRY - MEANS \pm 1 S.D. FROM FOUR PREDNISOLONE - TREATED LYMPHOMA PIGS, UP TO DAY 37 AND 2 PIGS THEREAFTER (MMol/LITRE)

DAYS FROM START OF DOSING	SODIUM	POTASSIUM	CHLORIDE	CALCIUM	MAGNESIUM	PHOSPHATE
- 25	133.0 \pm 5.7	5.00 \pm 0.40	89.0 \pm 2.2	2.55 \pm 0.17	0.84 \pm 0.05	2.27 \pm 0.14
- 19	135.5 \pm 3.3	5.20 \pm 0.40	91.0 \pm 1.4	2.66 \pm 0.05	0.86 \pm 0.07	2.21 \pm 0.05
- 12	143.0 \pm 2.1	4.45 \pm 0.34	91.0 \pm 1.6	2.49 \pm 0.33	0.84 \pm 0.04	1.59 \pm 0.07
- 5	137.0 \pm 4.1	4.50 \pm 0.35	90.5 \pm 3.0	2.65 \pm 0.15	0.83 \pm 0.07	2.15 \pm 0.33
Start of Dosing						
2	148.0 \pm 0	4.45 \pm 0.37	91.5 \pm 1.9	2.60 \pm 0.20	0.96 \pm 0.07	2.43 \pm 0.30
9	134.5 \pm 1.7	4.70 \pm 0.70	94.0 \pm 1.3	2.74 \pm 0.15	0.84 \pm 0.02	2.09 \pm 0.20
16	138.0 \pm 1.6	4.25 \pm 0.50	96.5 \pm 3.2	2.61 \pm 0.07	0.92 \pm 0.05	2.10 \pm 0.18
23	146.0 \pm 2.6	5.45 \pm 0.65	91.0 \pm 6.9	3.04 \pm 0.37	0.98 \pm 0.13	1.90 \pm 0.34
30	148.0 \pm 3.3	5.15 \pm 1.40	89.8 \pm 1.7	2.57 \pm 0.22	0.86 \pm 0.07	1.77 \pm 0.17
37	141.5 \pm 5.0	5.03 \pm 0.72	91.3 \pm 5.9	2.64 \pm 0.08	0.89 \pm 0.04	1.79 \pm 0.33
End of Dosing						
44	147.5 \pm 3.5	4.60 \pm 0.14	94.5 \pm 0.7	2.71 \pm 0.04	0.75 \pm 0	2.34 \pm 0.26
51	151.0 \pm 4.2	4.50 \pm 0	96.0 \pm 0	2.68 \pm 0	0.71 \pm 0.01	2.16 \pm 0.40
58	146.5 \pm 3.5	5.50 \pm 0.14	93.0 \pm 2.8	2.81 \pm 0	0.71 \pm 0.04	2.41 \pm 0.32
65	144.0 \pm 5.7	5.40 \pm 0.14	96.5 \pm 2.1	2.85 \pm 0.07	0.71 \pm 0.04	2.56 \pm 0.20
72	150.0 \pm 5.7	4.45 \pm 0.07	92.0 \pm 1.4	2.65 \pm 0.07	0.73 \pm 0.09	2.20 \pm 0

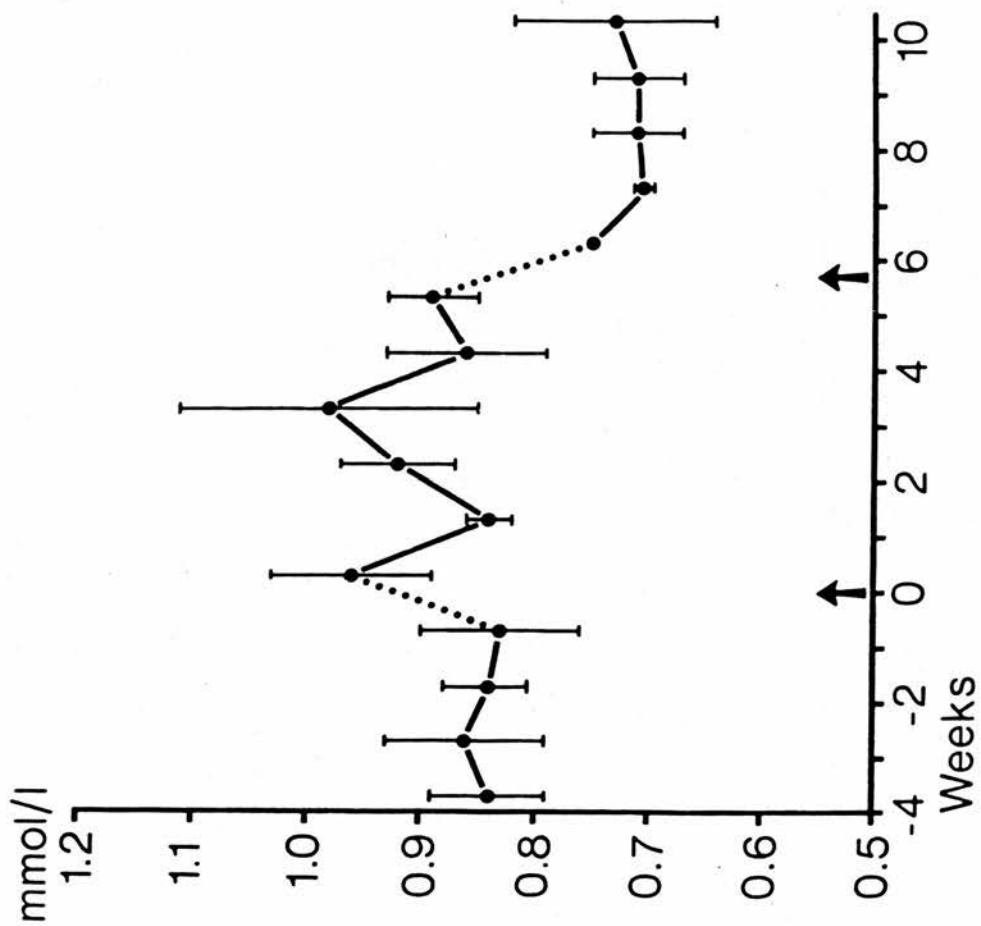


FIGURE 8

Serum magnesium levels (means \pm 1 S.D.) of lymphosarcoma cases. Arrows indicate beginning and end of prednisolone treatment.

was rather wider than our figures for the normal range (142 to 148 mmol/l) but Baetz and Mengeling (1971) gave a figure of 131 mmol/l as normal for adult animals. This is closer to the results obtained during the pre-treatment period. Serum chloride levels also tended to be lower than normal (92 to 110), again particularly during the pretreatment period, but serum calcium and inorganic phosphate were mostly within the normal range (2.3 to 2.8 and 1.71 to 3.10 mmol/l respectively).

Serum Proteins

Previous experience has shown that serum protein levels in untreated lympho/^{sarcomatous} pigs vary considerably between animals, and within animals from time to time, but there is usually a marked increase in globulins, due to an increase in immunoglobulin factor IgG (Imlah and McTaggart, 1977), whereas serum albumin tends to be low.

Total serum globulin values were obtained by subtraction of serum albumin from total protein. Measured results are shown in Appendix I and mean figures \pm 1 S.D. in Table 18. Figure 9A shows mean values only of serum albumin and total serum globulin. During treatment, mean serum globulin fell sharply, reaching a nadir of 21.0 ± 5.2 g/l during the fourth week. At the same time, mean serum albumin rose, reaching a peak during the fourth week, of 46.0 ± 3.9 g/l. Total protein levels were not much affected.

These effects were exaggerated when the albumin/globulin ratios were calculated. See Figure 9B.

TABLE 18

SERUM PROTEINS OF PREDNISOLONE-TREATED LYMPHOSARCOMA PIGS.
 MEANS \pm 1 S.D. FROM 4 PIGS UP TO DAY 37 AND 2 PIGS THEREAFTER (g/l)

DAYS FROM START OF DOSING	TOTAL PROTEIN	SERUM ALBUMIN	SERUM GLOBULIN (BY DIFFERENCE)
- 25	60.5 \pm 4.4	23.0 \pm 4.3	37.5 \pm 3.4
- 19	74.0 \pm 2.8	23.0 \pm 5.2	50.75 \pm 7.2
- 12	70.0 \pm 3.3	24.0 \pm 4.6	45.5 \pm 7.0
- 5	62.5 \pm 2.6	24.0 \pm 3.6	38.8 \pm 7.0
Start of Dosing			
2	69.0 \pm 4.3	26.5 \pm 1.7	42.8 \pm 5.1
9	64.0 \pm 8.5	33.0 \pm 1.6	31.6 \pm 6.2
16	67.5 \pm 3.0	37.0 \pm 3.7	30.5 \pm 4.1
23	67.0 \pm 8.9	46.0 \pm 3.9	21.0 \pm 5.2
30	68.3 \pm 4.5	38.5 \pm 2.1	29.8 \pm 4.7
37	62.0 \pm 8.0	37.5 \pm 6.1	24.5 \pm 6.8
End of Dosing			
44	66.5 \pm 7.8	37.5 \pm 0.7	29.0 \pm 8.5
51	67.0 \pm 7.1	37.0 \pm 1.4	30.0 \pm 5.7
58	58.0 \pm 1.4	32.5 \pm 0.7	25.5 \pm 0.7
65	63.0 \pm 4.2	30.0 \pm 2.8	33.0 \pm 1.4
72	66.5 \pm 5.0	31.0 \pm 1.4	35.5 \pm 3.5

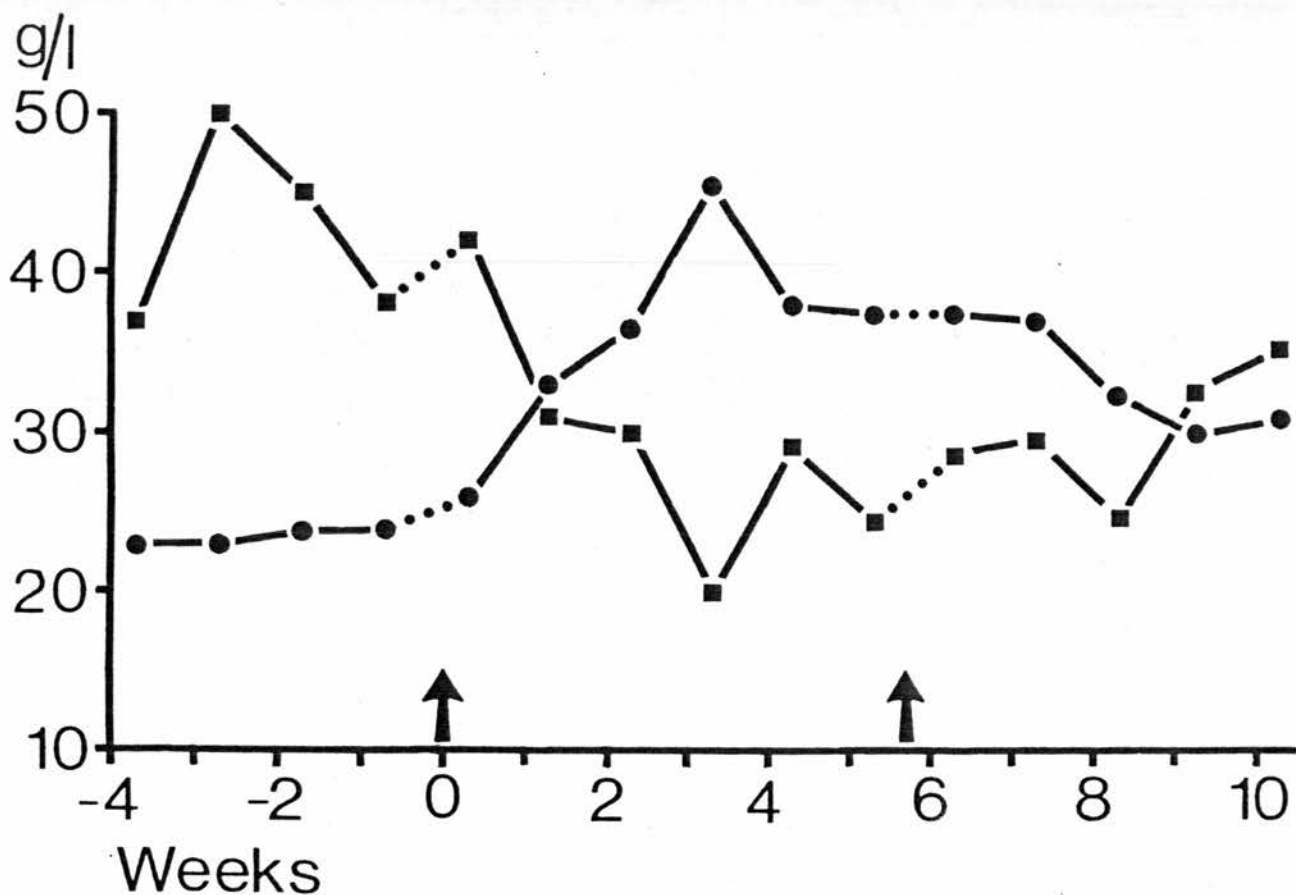
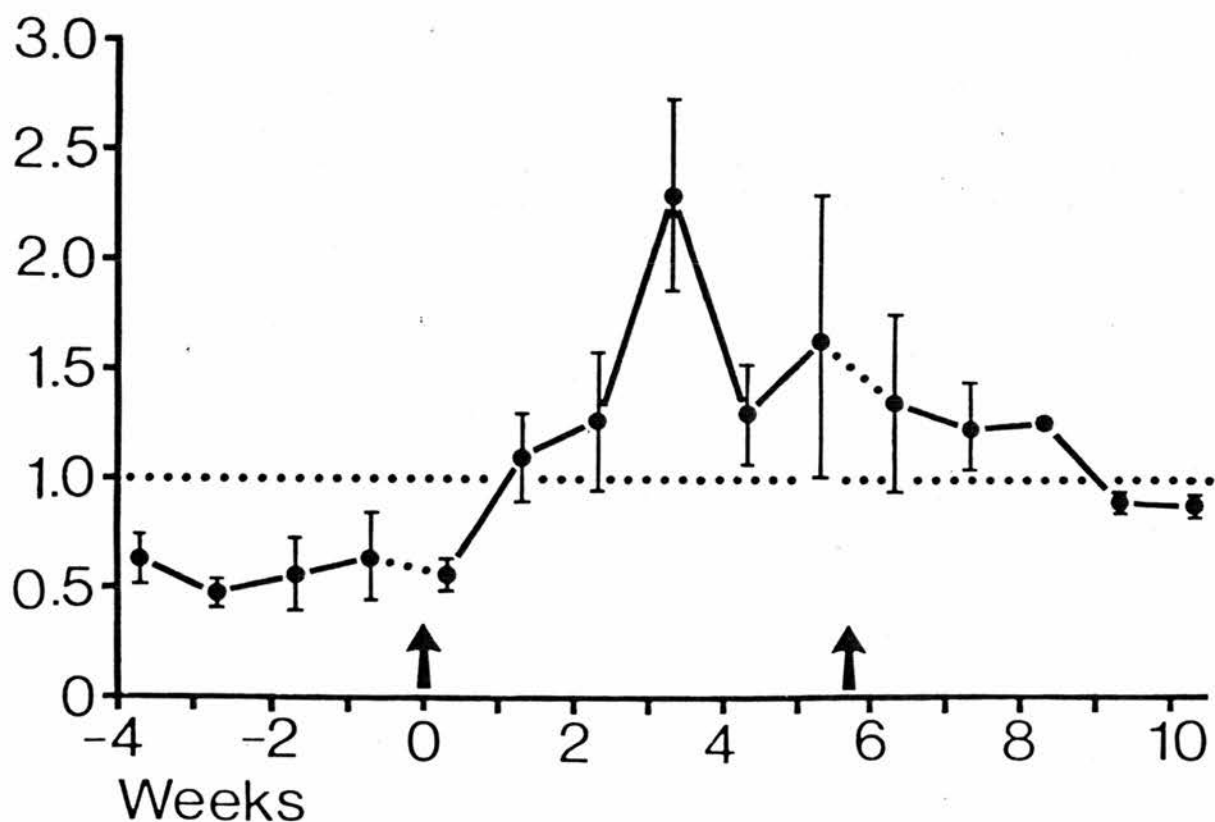


Figure 9A (above)
Mean serum albumin (● — ●) and globulins (■ — ■) and

Figure 9B (below)

Albumin/globulin ratios (means + 1 S.D.) of lymphosarcoma cases. Arrows indicate beginning and end of prednisolone treatment.



Post-Mortem Findings - Gross Pathology

Pig 3630 - killed at end of treatment.

This was a well-developed, well-nourished carcass.

The thymus consisted of a sheet 1cm wide by 0.2cm thick throughout the whole length of the neck and in the anterior mediastinum.

There was about 50 ml of clear fluid in the pericardial sac but none in chest or abdominal cavity.

There were a few petechiae on the lungs.

The heart showed a wedge-shaped dry red area 2.5cm across, with several 0.2cm yellow dry foci centrally, on cut surface of a papillary muscle. The valves were normal.

The spleen was red and engorged. White pulp foci of 1mm diameter could be seen when the blood ran out.

The stomach was normal and full of food.

Small and large intestine, liver and kidneys showed no abnormalities.

The semimembranosus and semitendinosus muscles of the hind legs showed 3 to 4cm diameter irregular areas of fibrosis with some yellowish firm streaky areas with congested borders.

All lymph nodes showed a very firm, fawn cut surface with no distinguishable cortex and medulla but distinct fibrous trabeculae. The cervical, popliteal, gastric and splenic nodes showed haemorrhage in the medulla region.

The bone marrow of the femoral shaft was red and

cellular. Both ends of the long bone, the bodies of the lumbar vertebrae and wing of the ilium were also active.

Pig 3656 - killed at end of treatment.

This was a moderately developed, moderately nourished carcass, with fat present.

The thymus was a thin white sheet of 1 to 2 mm soft nodules, mostly fat.

There was no fluid in chest or abdominal cavity but about 30 ml of clear fluid was present in the pericardial sac.

The liver was slightly pale. Lobulation was not distinct.

Lungs, heart and kidneys showed no abnormalities.

The spleen was contracted. White pulp nodules of 1 mm or less were just visible on cut surface.

The stomach was full of food. The oesophageal region showed a 5 mm by 2 mm green raised necrotic ulcerated area and a 5mm wide, 12mm long depressed white scar. The remainder of the stomach was slightly flushed but normal.

The small intestine showed distinct corrugations in the duodenum, slightly nodular but without necrosis. Peyer's patches were not easily seen and the rest was normal. The mucosa of the caecum was finely nodular but the wall was not thickened. The colon was normal.

In the muscles on each side of the neck and in the semitendinosus and semimembranosus muscles there were

ill-defined areas of consolidation due to white fibrosis between and replacing muscle fibres. There were small groups of red and yellow dry 2mm foci in these areas.

The lymph nodes showed firm, fawn lobulated tissue with a hint of medulla. The iliac and popliteal nodes showed wider areas of congested medulla. The mesenteric and gastric nodes were mottled and greenish on cut surface, firm with no cortex and medulla distinguishable but some clear fluid cysts. The splenic lymph node was mainly red, and on cut surface was mottled deep red in a grey background.

The bone marrow of the shaft and epiphyses of the femur, vertebral bodies and ilial wing was red and active.

Pig 3637

This was a well-developed, moderately nourished carcase.

The thymus was relatively large compared with untreated cases of comparable age with pale, fawn-coloured lobules.

There was no fluid in chest or abdominal cavity, but there was about 20ml of clear fluid in the pericardial sac.

The liver was of moderate size with slightly prominent perilobular pallor, either fibrosis or tumour.

Lungs, heart, kidneys and stomach showed no abnormalities.

The spleen was enlarged. Along the hilus, there was a series of 2cm nodules. The borders were curled

over to the non-hilar surface in some regions. The cut surface was red and cellular with 2 to 3mm pale pink foci scattered throughout. The nodules extended into the substance. They measured 1.5 to 2cm and were composed of pink tissue with some ill-defined white areas. They had a sharp border but no capsule.

The duodenum showed marked corrugation along its long axis, 5 cm wide, raised 3 to 4 mm, closely resembling adenomatosis, but on fixation clearly due to white tissue under the mucosa. The corrugation was present for the first 35 cm, then the intestine was normal until the ileum, where Peyer's patches were slightly thickened. The large intestine was normal.

There was no evidence of muscle lesions.

All lymph nodes were enlarged, and on cut surface showed a flat field of fawn cellular tissue with a faint nodularity but no medulla, except in the internal and external iliacs and popliteal which showed a 1mm wide brown medulla and white cortical nodules.

The splenic lymph node showed a red patchy haemorrhage in the medulla region.

The bone marrow of the midshaft of the femur was yellow/red mottled and translucent. It floated in formalin.

Pig 3652

This was a moderately developed, pot-bellied animal, with little fat present.

The thymus was very small, composed of pale fawn lobules.

There was no fluid in chest or abdominal cavity but about 20ml of clear fluid was present in the pericardial sac.

Lungs, heart, liver, kidney and stomach showed no abnormalities.

The spleen was enlarged but there were no nodules along the hilus. The edges curled over onto the non-hilar surface and were attached by adhesions in some regions. There were two infarcts near the dorsal extremity approximately 3cm by 5cm extending from the border as far as the hilus. They were yellowish red in colour. Nodules of white pulp were present but less distinct than in 3637. After fixation the infarcts were more distinct as yellow, dry necrosis with a reddish border in some areas.

The duodenum and remainder of the alimentary tract were similar to 3637 but the corrugation was less marked.

All carcase lymph nodes were enlarged and showed a flat field of fawn cellular tissue with no evidence of medulla except the external and internal iliac nodes which showed a slight pink medullary pattern. The medullary region of the splenic node showed very marked haemorrhage.

Histological Examination

Tissues from pigs killed at the end of treatment

In the pigs killed at the end of treatment, the lymph nodes showed a depletion of cells in the paracortical region, and the few nodules found had reverted

to primary cell structures. There were a few probable tumour cells in the paracortical tissue but none was in mitosis. The inguinal nodes were unique, in that one pole showed lymphoid depletion but the other pole was replaced by tumour.

The thymuses were reduced to lobules of adipose tissue with occasional epithelial cells and groups of up to 50 lymphocytes.

The white pulp of the spleen showed no nodules and the red pulp was depleted of cells.

In bone marrow samples from these pigs no tumour cell masses were found. There were more haemopoietic cells and less adipose tissue than normal. The femoral shaft marrow contained haemopoietic tissue. It is normally a fat store in this age of pig.

In contrast to other lymphoid sites, the lymphoid tissue in the stomach, Peyer's patches and caecum showed large centres of proliferating cells, particularly in pig 3656.

Tissues from pigs killed at the end of the experiment

As in typical, advanced, untreated cases of lymphosarcoma, the lymph nodes of these animals showed replacement of normal structure almost completely by tumour cells, except for a few small nodules.

The thymuses showed recovery of normal cortical and medullary structure, resembling that seen in the prednisolone-treated normal pigs, but the more rapidly re-

growing tumour had caused some thymus suppression in pig 3652.

The spleens resembled those of typical late-stage untreated lymphosarcoma. The tumour had regrown, nodules had reformed and extramedullary haemopoiesis had developed.

Bone marrow samples showed an increase in normoblast foci, a reduction in megakaryocytes and large islands of tumour cells. The marrow of the femoral shaft was haemopoietic and neoplastic in both pigs.

Tumour lymphocyte infiltration was present in some organs e.g., in the kidney and in the ovarian stroma, and there were proliferating tumour masses in the periportal areas of the liver and peribronchial areas in the lung.

Lymph node and organ weights

See Appendix I for absolute weights in grams. Table 19 shows the relative weights of lymph nodes and organs, compared with those of a normal litter-mate, and 17 untreated cases (mean and range).

From the results of the pigs killed at the end of treatment it can be seen that prednisolone dramatically reduced the relative weights of the splanchnic nodes (mesenteric and colonic, gastric and splenic and bronchial nodes) to below the minimum value of the 17 untreated cases, but they were still about three times larger than those of the untreated normal pig. The

TABLE 19.

RELATIVE WEIGHTS OF LYMPH NODES AND ORGANS (cg/kg body weight) OF INDIVIDUAL LYMPHOMA PIGS TREATED INTRAMUSCULARLY WITH PREDNISOLONE, COMPARED WITH AN UNTREATED NORMAL LITTERMATE OF TWO OF THEM, AND 17 UNTREATED CASES OF SIMILAR AGE

PIG NUMBER	NORMAL UNTREATED 3654	KILLED AT END OF TREATMENT 3630	3656	KILLED AT END OF EXPERIMENT 3637	3652	MEAN (RANGE) OF 17 UNTREATED LYMPHOSARCOMA CASES
Age at death (days)	221	237	219	272	244	231 (200 to 280)
Lymph Nodes :						
Mesenteric and Colonic	267	764	565	5368	2836	4177 (646 to 9906)
Gastric and Splenic	28	230	336	1687	1115	1960 (740 to 3210)
Bronchial Group	7	25	23	77	84	217 (38 to 919)
Total Splanchnic	302	1019	924	7132	4035	6261 (1662 to 12670)
Head Group	25	61	62	143	237	163 (45 to 286)
Iliac Group	7	13	11	38	75	36 (11 to 67)
Prescapular	2	5	4	12	15	15 (4 to 47)
Precrural	3	13	7	27	35	23 (7 to 39)
Inguinal	8	25	20	47	64	51 (18 to 156)
Popliteal	1	3	3	6	26	11 (2 to 16)
Total Superficial	46	120	107	273	452	290 (93 to 476)
Thymus	100	31	21	67	42	18 (0 to 67)
Spleen	373	500	242	1250	1197	2156 (1030 to 7800)
Liver	2193	5069	4194	4711	5607	6288 (3120 to 9270)
Kidney	167	325	209	263	262	331 (180 to 443)
Adrenal	3	4	4	7	5	11 (5 to 28)

weights from the pigs which were allowed to relapse approximated to the mean values of the untreated cases. Similar, but less spectacular effects were observed in the superficial nodes, individually and as a group.

The relative weights of the thymuses in the relapsed pigs were about twice as great as those of the pigs killed at the end of treatment. All were above average for untreated cases, in which the thymus is usually suppressed, but none was as large as that of the untreated normal pig.

The effects on the splenic relative weights were similar to those on the lymph node weights. Liver relative weights were similar to untreated cases and greater than that of the normal pig.

ORAL THERAPYExperiment BMATERIALS AND METHODS

Two uncastrated male pigs were used. Lymphosarcoma had been diagnosed when they were 6 weeks old. One (141) was 5 months old and the other (67) 7 months old at the start of the experiment.

The pigs were housed in separate pens. No supportive therapy was given, since the pigs were in reasonably good body condition.

Methods were as in Experiment A except that pre-treatment blood-sampling was only carried out for 2 weeks. Serum electrolytes were not measured, because of the expense involved, but the same haematological parameters plus serum total protein and albumin, and blood urea were recorded.

Prednisolone tablets (Evans Medical), 5mg, were crushed and added to food once daily, 5 days per week for 6.5 weeks, at the same dose rate of 4mg/kg. As before, weighing, blood-sampling and detailed clinical examinations with lymph node measurements were carried out once weekly.

Sensitisation to DNFB was carried out at -4 weeks, and testing at -2 weeks and 6 weeks. One pig (67) was destroyed at the end of treatment, by pentobarbitone overdose and autopsy was carried out. The remaining pig (141) was given a $\frac{1}{2}$ dose, $\frac{1}{4}$ dose and $\frac{1}{8}$ dose over 3

days and then treatment was discontinued. It was examined and blood-sampled weekly for a further 6 weeks, after which it was skin-tested, destroyed and autopsied.

RESULTS

Clinical Examinations (See Clinical Records, Appendix I)

Before treatment, both pigs were lethargic but had reasonably good appetites. No difficulties were encountered with dosing.

Within the first two weeks of treatment, both animals had an attack of severe diarrhoea. Although appetite was depressed both pigs could still be persuaded to eat. They were treated with neomycin sulphate ("Neobiotic", Upjohn), 1g each per day mixed with food for 5 days, during which time both recovered. Two days after neomycin was stopped, 141 again developed severe diarrhoea. This time, it refused to eat. A sample was submitted for bacteriology, and chloramphenicol ("Ertilen", Ciba-Geigy 150mg/ml), 1ml/10kg, was administered intramuscularly for 5 days. By the following day, a marked improvement was noticed. Four days after the injections were stopped 141 again developed severe diarrhoea. Laboratory tests failed to demonstrate the presence of accepted bacterial pathogens, but very large numbers of Balantidium coli were seen in faecal smears. Though this is not normally pathogenic in pigs, treatment with 20ml of a mixture containing sulphadimidine, dihydrostreptomycin sulphate and kaolin ("Streptaquine sulpha", Elanco) was administered by mouth for 5 days. Once again improvement was rapid. No further trouble with this animal was encountered until just before it was destroyed at the end of the experiment,

TABLE 20

PREDNISOLONE ORAL THERAPY

Lymph node measurements - mean products of lengths and breadths/of inguinal and precrural nodes. in mm.

Week	PIG 67		PIG 141	
	Inguinal	Precurral	Inguinal	Precurral
0	1275	632	2013	744
Start of Dosing				
1	1392	452	1104	400
2	1172	414	1132	336
3	965	359	912	371
4	900	Very Small	757	Very Small
5	758	Very Small	858	Very Small
6	607	Very Small	690	Very Small
7	DEAD	DEAD	775	Very Small
8			1063	Very Small
9			Not Measured	Not Measured
10			1362	Very Small
11			1936	870
12			2016	1202

when it again developed diarrhoea. Pig 67 also had two further attacks of diarrhoea during treatment. It was also treated with "Streptaquaine" and recovered.

Rectal temperatures were within the normal range throughout the experiment.

As in Experiment A, there was a shrinkage of superficial lymph nodes during prednisolone treatment (See Table 20). In pig 141, this was very rapid - 45% in the inguinal nodes and 46% in the precrural nodes by the end of the 1st week of treatment. By the 3rd week on prednisolone, the decrease in the mean product of lengths and breadths of the inguinals was 55% and 50% in the precrurals. In pig 67, the decrease in size of the nodes was not quite so rapid and by the 3rd week, the inguinal nodes only showed a decrease of 24%. However the precrurals were decreased by 43%. By the 5th week, the inguinals were reduced by 52%.

As before, when treatment was stopped, the lymph nodes rapidly increased again, until by 6 weeks after the end of treatment, they were as large as they had been at the beginning of the experiment.

TABLE 21.

Body Weight Gains - Oral Prednisolone Therapy

Pig No.	Wt. at start of treatment (kg)	Wt. at end of treatment (kg)	Gain	Wt. at end of expt. (kg)	Gain
67	27	36.5	9.5	Dead	-
141	28	40	12	57	17

Weight Gains (See Table 21).

Compared with the results in Experiment A (see Table 5 page 128) weight gains during treatment were better, despite the attacks of diarrhoea, than with intramuscular therapy. As in Experiment A, weight gain increased after treatment was stopped in pig 141.

TABLE 22

Pig No.	At sensitisation	Pre-treatment	Last week of treatment	6 weeks post-treatment
67	1	0	0	Dead
141	2	0	0	0

DNFB Skin Test Results

Before treatment started both pigs were anergic to DNFB, and this situation did not change during the experiment. (See Table 22 above).

Haematology

(See Appendix I, Tables 46, 47, 52 and 53).

Total WBC counts fell in both pigs during treatment. If the absolute mononuclear cell and neutrophil counts are examined, the results are spectacular (See Figure 10). Before treatment started, both animals had low neutrophil counts (3.5 and $1.6 \times 10^9/l$) and high mononuclear cell counts (23.1 and $25.7 \times 10^9/l$) with a very high percentage of these being morphologically abnormal. Within 1 week of starting oral prednisolone therapy,

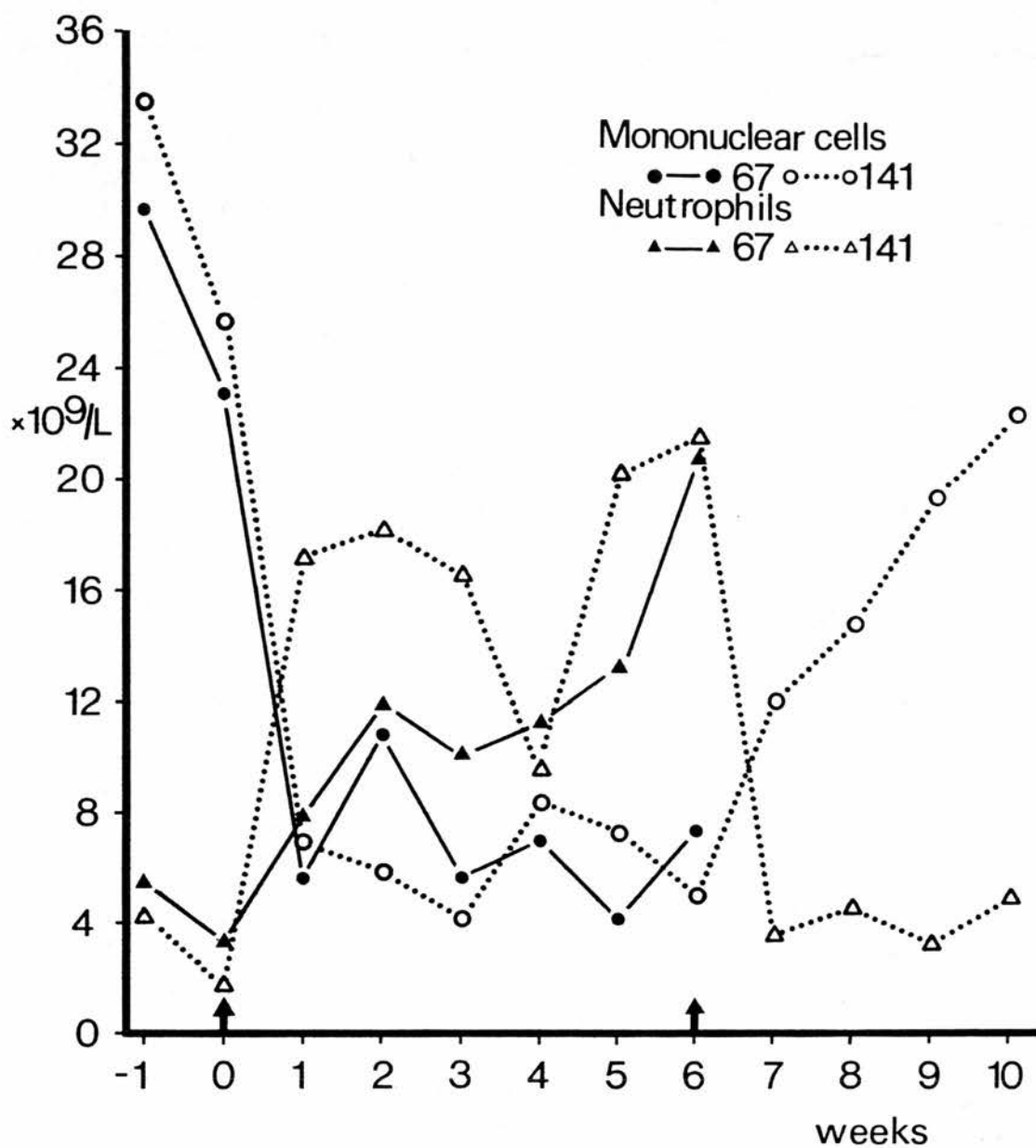


FIGURE 10

Absolute mononuclear and neutrophil counts of two lymphosarcoma cases treated orally with prednisolone. Arrows indicate beginning and end of treatment.

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(cf flav.)
5 HG

neutrophil counts rose and mononuclear counts fell, and abnormal cells almost disappeared from blood smears. Within 1 week of stopping treatment in 141, the neutrophil count fell to pretreatment level, and the mononuclear cell count started to rise, as abnormal cells reappeared.

As in Experiment A, thrombocyte counts rose during therapy, from $154 \times 10^9/l$ to a peak of $404 \times 10^9/l$ by week 2 in pig 67, and from $65 \times 10^9/l$ to a peak of $562 \times 10^9/l$ in week 4 in pig 141. After treatment was withdrawn, the count in 141 fell again to $119 \times 10^9/l$ (See Appendix I).

At the beginning of the experiment, both pigs were anaemic. Pretreatment RBC counts, PCV and Hb were $3.45 \times 10^{12}/l$, 0.20L/l and 7.1g/dl in pig 67 and $3.72 \times 10^{12}/l$, 0.215 l/l and 7.5g/dl in pig 141. As in Experiment A, red cell parameters increased during the treatment period. In pig 67, the RBC count rose only to $4.85 \times 10^{12}/l$, and Hb to 9.9 g/dl, in the last week of treatment, but PCV improved markedly, reaching 0.32 l/l in the 5th week. In pig 141, there was a rapid rise in all red cell parameters, and as in Experiment A, the improvement lasted for a short time after treatment was stopped. At this time, its red cell picture was almost normal (RBC - $6.24 \times 10^{12}/l$, PCV - 0.38 l/l, Hb - 12.6 g/dl). After this, however, it became progressively more anaemic again (See Appendix I).

MCV increased slightly during treatment in both animals.

Serum Proteins (See Appendix I)

Once again, the results were very similar to those obtained with parenteral prednisolone therapy, namely a fall in total serum globulin and a rise in serum albumin, which was reversed in pig 141 when treatment was withdrawn.

The albumin/globulin ratios increased, from pre-treatment values of 0.69 and 0.61 to peak values of 2.09 in the 3rd week in pig 67, and 1.97 by the end of the 1st week in pig 141. As 141 relapsed, the albumin/globulin ratio fell again to 0.59.

Blood Urea

Blood urea levels stayed within the normal range in both animals throughout the experiment.

Post Mortem Findings - Gross Pathology

Pig 67 - killed at end of treatment.

This was an anatomically normal carcase, though rather small for its age and slightly pot-bellied. There was plenty of fat at all normal body sites.

At the site of the thoracic and cervical thymus there was a structure which was lobulated and white, but which was probably all fat as it was soft and greasy and floated in formalin.

Head and neck and lungs showed no abnormality.

There was a trace of serosanguinous fluid in the

chest cavity and about 20ml in the pericardial sac. The thoracic surface of the rib muscles and ribs showed a white nodular thickening, especially ventrally and towards the diaphragm, which mostly appeared to be due to deposition of fat but in one area there was distinct fibrosis with adhesion to the adjacent part of the diaphragm. The epicardium of the right ventricle showed numerous small organising fibrin tags, also present on the left ventricular side but only towards the tip. The muscle and valves were normal.

There was no evidence of fluid in the abdominal cavity and none of the organs was excessively large, therefore the pot-bellied appearance was due to slackness of the abdominal muscles.

The spleen was slightly enlarged but with no curving over of the borders. The cut surface was red and cellular and no blood exuded from it. The white pulp was visible.

The liver was slightly pale but of normal consistency and the lobulation was not distinct.

The kidneys, bladder and adrenals showed no abnormality.

The oesophageal region of the stomach showed slight yellow thickening especially near the mucous membrane junction but no true ulceration. The cardiac region showed a coating of mucus in which tags of black altered blood were visible. These arose from irregular small

cracks in the mucous membrane, which, on cut surface, extended 1mm into the underlying mucosa, never more than halfway through it. The fundic region showed four nodules 5mm in diameter slightly raised above the surface, probably due to lymphoid aggregates under the mucosa. The pylorus was normal.

The duodenum, small intestine, large intestine and pancreas were normal. The small intestine contained two adult ascarid worms.

The testes were of normal size, and smears made from them showed numerous adult and nearly adult sperm.

On opening the skull, haemorrhage could be seen under the meninges, and on cut surface this was up to 5mm thick over the cerebrum and extending round ventrally under the base of the brain, but it did not appear to involve the cerebellum. On seeing this the attendant remarked that the animal had been behaving peculiarly the day before it was destroyed, but there had been no obvious trauma and there was no subcutaneous bruising. The bones of the skull appeared normal.

All the lymph nodes showed half and half white nodular cortex and buff medulla. Some of the gastric nodes, the iliacs and splenics showed areas of haemorrhage in the medulla. There were chalky white foci in the inguinal and cervical nodes. The mesenteric nodes were smaller than the colonic nodes.

The bone marrow of the femur appeared to show half and half white fat and red active marrow.

Pig 141 - killed 6 weeks after end of treatment.

This was a well-developed, well-nourished car-case with plenty of normal fat.

The thymus was large with soft, white lobules, both in the neck and in the chest.

There was about 50ml of clear fluid in the pericardial sac but none in the chest or abdomen.

Head, neck and heart showed no abnormality. The lungs were partially collapsed, probably due to post-mortem change but there were no focal lesions.

The spleen was grossly enlarged. The cut surface showed ill-defined 2 to 3mm nodules, probably enlarged white pulp. Only a little blood flowed out. No tumour nodules were visible along the hilus.

The liver lobulations were more distinct than normal, but colour and consistency were normal.

The kidneys, bladder and adrenals showed no abnormality.

The fundus of the stomach was slightly thickened and flushed. The duodenum showed prominent corrugation due to thickening of the wall by up to 1cm of white cellular tissue. Cryostat sections showed lymphocytes causing most of the infiltration and there was no evidence of adenomatosis. Peyer's patches showed up as thickening of the mucosa by white tissue.

The mucosa of the caecum and colon showed small nodules.

The right testis was descended and was of normal shape and size with a normal cut surface. The epididymal tubules were large and filled with a milky fluid. This contained large numbers of very active sperm under phase contrast and when stained eosin nigrosin only a few stained pink i.e. were dead. Many however showed cytoplasmic remnant part way down the tail.

The left testis was in the abdominal cavity at the internal inguinal ring. It was much smaller in size (weight 48g compared with 232g for the right). The epididymal tubules were small and empty.

The seminal vesicles were moderately enlarged, though not the size of a fully-developed boar, and on cut surface they were more congested.

All the lymph nodes were enlarged and on cut surface were lobulated, with an almost flat field of white or buff tissue with no visible medulla. Only the splenic lymph node showed the medulla outlined by haemorrhage.

The last mesenteric and left bronchial node showed several cystic structures containing straw-coloured fluid.

The bone marrow of the femur was red mottled with white and floated heavily in formalin - probably a mixture of congestion and activity, throughout the length of the shaft.

Relative weights of lymph nodes and organs

Table 23 shows the relative weights calculated from the absolute weights shown in Table 60, Appendix I. These are compared with those of the normal untreated pig from Experiment A and 17 untreated cases. It can be seen that the results are similar to those in Experiment A, showing a marked reduction in the weights of the lymph nodes to below the minimum value for untreated cases in 67. The superficial nodes in the relapsed pig 141 were heavier than the mean value for untreated cases but the splanchnic nodes were lighter. The thymus weight in 141 was greater even than that of the normal pig.

TABLE 23

RELATIVE WEIGHTS OF LYMPH NODES AND ORGANS (cg/kg body weight) OF LYMPHOMA PIGS TREATED ORALLY WITH PREDNISOLONE, COMPARED WITH AN UNTREATED NORMAL PIG AND 17 UNTREATED CASES OF SIMILAR AGE.

PIG NUMBER	NORMAL UNTREATED	KILLED AT END OF TREATMENT	KILLED AT END OF EXPERIMENT	MEAN (RANGE) OF 17 UNTREATED CASES
Age at death (days)	3654	67	141	
	221	267	236	231 (200 to 280)
Lymph Nodes :				
Mesenteric and				
Colonic	267	986	3667	4177 (646 to 9906)
Gastric and Splenic	28	132	1124	1960 (740 to 3210)
Bronchial Group	7	18	119	217 (38 to 919)
Total Splanchnic	302	1136	4900	6261 (1662 to 12670)
Head Group	25	43	141	163 (45 to 286)
Iliac Group	7	10	31	36 (11 to 67)
Prescapular	2	3	7	15 (4 to 47)
Precrural	3	7	118	23 (7 to 39)
Inguinal	8	16	43	51 (18 to 156)
Popliteal	1	3	9	11 (2 to 16)
Total Superficial	46	82	349	290 (93 to 476)
Thymus	100	34	136	18 (0 to 67)
Spleen	373	487	2123	2156 (1030 to 7800)
Liver	2193	5414	4754	6288 (3120 to 9270)
Kidney	167	299	281	331 (180 to 443)
Adrenal	3	6	5	11 (5 to 28)

DISCUSSION

Soon after the first reports of the successful use of corticosteroids in human cancer, Bloom (1952) used corticosteroids in cases of mast cell tumour, and Clover (1958), Agresti (1959) and Jacquier (1963) described good results in the therapy of lymphosarcoma in dogs. In one small experiment, successful steroid treatment of chickens paralysed with Marek's disease was reported (Foster and Moll, 1968). Reichel (1962) treated two pigs in the terminal stages of lymphosarcoma with prednisolone, and showed that the white blood cell counts fell although the pigs subsequently died.

Prednisolone was a logical choice therefore, for the first therapy trial of hereditary porcine lymphosarcoma. The dose level of 4mg/kg had produced no serious adverse effects in the normal pigs, and was considered likely to be safe in these pigs, which were in poor condition as a result of the advanced stage of the disease.

The results of the treatment were dramatic in several respects. It became obvious very soon after the start of the treatment, that the tumour was being destroyed as shown by the rapid shrinkage of the superficial lymph nodes and the decreasing numbers of abnormal mononuclear cells seen when performing differential WBC counts. The percentage reduction in circulating mononuclear cells was twice as great as that observed even

at the highest dose level (6mg/kg) in the normal pigs. This was in keeping with the observation by Schrek (1961) that leukaemic lymphocytes were more sensitive to corticosteroids than were normal lymphocytes. The suppression of the tumour was accompanied by an improvement in the fitness/^{vigour}and appetite of the pigs, even though their growth rate did not improve. The poor body weight gains during treatment and increases after prednisolone withdrawal were in keeping with the known growth-depressive effects of prednisolone (Myles and Daly, 1974). However, the weights recorded may have been affected to some extent by changes in tumour mass.

Although the average skin hypersensitivity reaction score of two in the pigs given prednisolone intramuscularly was not as high as would be expected in a normal untreated pig, it was higher than that observed in untreated lymphosarcoma/^{tous}pigs (unpublished data). These reactions suggest that prednisolone had a slightly beneficial effect on the cell-mediated response, unlike that observed in the normal pigs, where the effect was to suppress the hypersensitivity reaction. However this beneficial effect was not apparent in the pigs given prednisolone orally.

A major difference between the intramuscular and oral therapy was the effect on neutrophil counts. In the pigs given prednisolone intramuscularly, there was a slight rise in the neutrophil counts, especially during the 5th week, but the counts fell again at the end of

treatment. As in the experiment using normal pigs, this did not correspond with the marked neutrophilia reported in the literature. In the pigs given oral prednisolone, however, there was a much more pronounced rise in the neutrophil counts which lasted until the end of treatment. These two animals suffered from severe diarrhoea during the treatment period, whereas the four pigs given intramuscular prednisolone did not. Although no bacterial pathogens were isolated, it is possible that the prednisolone, or the immunosuppression produced by the lymphosarcoma, reduced the pigs' resistance to a normally harmless protozoan inhabitant of the large intestine, Balantidium coli, and that the neutrophilia was a response to the diarrhoea.

Red cell pictures and thrombocyte counts improved greatly during prednisolone treatment and the improvement in the red cell pictures continued into the second week after treatment was discontinued. This might have been due to a stimulating effect of prednisolone on early red cell precursors in the marrow, so that the resulting increased population of maturing cells in the "pipeline" continued to appear in the blood. The destruction of tumour in the marrow would also contribute to an improvement in the red cell and thrombocyte pictures by allowing previously suppressed precursor cells to regenerate.

It was shown in the experiment using normal pigs that prednisolone produced no effects on serum electrolyte levels. However, the upward fluctuations of serum magnesium observed in Experiment A, may have been due to release of the element from damaged malignant cells, and this has been recorded during treatment of human leukaemia patients by Ilicin (1971). The reduction to subnormal levels after the end of treatment in Experiment A might have been associated with increased uptake of the element from the blood stream by the rapidly regrowing tumour. It might be expected that serum potassium levels would alter in the same way as magnesium, but these remained unchanged, apart from the low level recorded in pig 3630 during the 3rd week. The clinical signs shown by this pig may have been due to low serum potassium levels, but at autopsy, it was discovered that it had a cardiac infarct and this would appear to be a more likely cause of the problem.

In the experiment using normal pigs there was a rise in serum albumin and a fall in serum globulins. This observation was confirmed in both therapy experiments and the effects were even more pronounced in the lymphosarcoma/^{tous}pigs. The reduction in globulins may have resulted from corticosteroid lympholysis, primarily of tumour cells which were producing an excess of gamma globulin. However it has not yet been

demonstrated that the high level of gamma globulin found in pigs with hereditary lymphosarcoma is produced by the tumour cells. Indeed, these appear to be neither T nor B cells (unpublished data). It may be that the gamma globulin came from normal B lymphocytes in response to the presence of the tumour. These cells were then lysed by the corticosteroid, or reduced their production of globulin as the tumour disappeared, or both.

Increased serum albumin production would be helped by prednisolone-induced improvement in appetite, resulting in increased protein uptake, and improved utilisation following reduced interference with organ function by the tumour or its products.

The suspicion that prednisolone produced a nadir in total serum globulins before the end of treatment in the normal pigs was confirmed in the lymphosarcomatous pigs, in which it was accompanied by a peak in serum albumin. A similar nadir in the mononuclear cell counts and peaks in neutrophil and thrombocyte counts, in Experiment A during the 5th weeks of treatment, suggest that after this time the effects of prednisolone began to decline. Rosenthal et al., (1951) stated that remissions in lymphoid neoplasia with ACTH or cortisone were of short duration. This waning of effect may occur because of suppression of the hypothalamic-pituitary-adrenal axis. The mean relative weight of

the adrenal glands of pigs killed at the end of treatment in Experiment A was only two-thirds that of the pigs which were allowed to relapse. The relative weights of the adrenal glands of all the treated pigs were much less than the mean weight for untreated lymphosarcoma pigs.

In the experiment using normal pigs no stomach lesions were found. In the therapy experiments, two out of the six treated pigs had gastric ulceration. In 3656 the lesion was in the usual site, but 67 showed cracks in the mucosa of the cardiac region, which may have been caused by prednisolone administration.

The histology results revealed that tumour growth was suppressed to different degrees in different sites in the body, and even in a single organ, e.g. in the inguinal node only localised suppression was achieved. It is impossible to identify isolated non-dividing tumour cells by conventional histology, therefore it was difficult to state whether treatment completely destroyed all the tumour cells in an area or just reduced their number and mitotic rate, so that they were no longer detectable.

The finding of tumour infiltration in the ovaries of the pigs which were allowed to relapse was interesting, because this has never been observed in untreated cases Head et al., (1974).

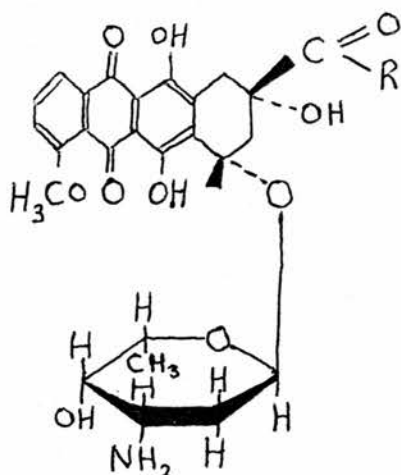
The results of these experiments show clearly that prednisolone treatment of hereditary lymphosarcoma produced at least a good partial remission, which corresponds well with the results obtained from the therapy of lymphoid tumours in man (See Introduction to this Chapter).

CHAPTER II

ADRIAMYCIN (DOXORUBICIN)INTRODUCTION

Adriamycin, which is also known by its synonym doxorubicin is an anthracycline antibiotic, a fermentation product of the fungus Streptomyces peucetius var caesius. Like the closely related drug daunorubicin, the adriamycin molecule has an aminoglucoside ring attached, and this sugar enables the drug to intercalate between adjacent base pairs in the double helical structure of DNA, inducing template disordering, thereby inhibiting the enzymes involved in DNA synthesis and RNA synthesis.

Formula of adriamycin and daunorubicin:-



Adriamycin $-R = \text{CH}_2\text{OH}$

Daunorubicin $-R = \text{CH}_3$

In vitro, adriamycin rapidly penetrates into cells and binds to nuclear structures, causing an immediate disturbance of mitosis. It inhibits prophase at low concentrations and at higher levels causes complete mitotic block.

Adriamycin and daunorubicin are most effective, therefore, during S-phase of the cell cycle, leading to accumulation of cells in late S or G_2 phase, but at

higher concentrations, all stages of the cell cycle are affected (Chabner, Myers and Oliverio, 1977).

Adriamycin hydrochloride is the compound used in chemotherapy. It is a stable, bright red powder, readily soluble in water or physiological saline. It is manufactured by Farmitalia (Farmitalia in Italy) who discovered it.

The in vitro antitumour activity of adriamycin was first assessed in various mouse tumours, such as Erlich ascites carcinoma, sarcoma/80, Oberling Guerin-Guerin carcinoma, a nitrosomethylurea-induced lymphosarcoma in C₃Hf mice and L1210 and P388 leukaemias. It produces an increase in survival time in all these tumours, frequently greater than that produced by daunorubicin. (DiMarco, 1972, Goldin, 1972). DiMarco also described the effects of therapy with adriamycin and daunorubicin in murine sarcoma virus (Moloney)-induced tumours in 16-day-old Swiss CD-1 mice. Normally untreated mice infected at this age developed the tumour but then showed a complete and permanent tumour regression. If treated with daunorubicin the tumour regressed but then recurred and killed the mouse.

This also happened to a lesser extent with adriamycin. When the drugs were administered before the infection, daunorubicin inhibited the natural tumour regression whereas adriamycin did not. These effects were attributed to differences in action of these compounds on the immune system, as well as to their action

against the tumour, disturbing the complex relationship between host, tumour and virus.

It is interesting that two such similar compounds can have quite different effects and this has been confirmed when these drugs have been used in human medicine. Daunorubicin is mainly used in the treatment of leukaemias, but adriamycin has been shown to be active against a wide range of tumours, either on its own or in combination with other drugs. (Bonnadonna, Monfardini, DeLena and Fossati-Bellani 1969, Bonnadonna, Monfardini, DeLena, Fossati-Bellani and Beretta, 1972, Middleman, Luce and Luce, Talley, Gottlieb, Baker and Bonnadonna Frei, 1971, O'Bryan, 1973, Tan, Etcubanas, Wollner, Rosen, Gilladoga, Showel, Murphy and Krakoff 1973, Blum and Carter, 1974 and Carter 1975). Examples of tumours against which adriamycin has been effective are malignant lymphomas, acute leukaemias, sarcomas and carcinomas of the breast, lung, thyroid, testis and urinary bladder, and Wilm's tumour in children.

Like most anticancer drugs however, its toxicity to the patient has been a major cause for concern.

The fate of adriamycin in the body after administration seems to vary considerably in different species. In mice, after intravenous dosage the drug was rapidly transferred to the tissues and reached high concentrations in all organs except the brain. Measurable concentrations in spleen, liver and kidneys were still present at 72 hours. High concentrations were found in the heart up to 24 hours after dosing (Arena, D'Allessandro, Dusonchet, Gebbia, Gerbasi, Sanguedolce and Rausa, 1972).

The same authors investigated the pharmacokinetics in the rabbit. The major route of excretion was via the bile, with practically none of the drug being excreted in the urine. They found that the drug was not absorbed by the gastro-intestinal tract. Yesair, Asbell, Bruni, Bullock and Schwartzbach (1972) stated that the drug was metabolised in hamsters but not in mice, rats or dogs. Rats excreted six percent of an intravenous dose in urine and eight percent in bile in 24 hours, and there were measurable concentrations of the drug in kidney, heart, liver and small intestine for 48 hours, higher than concentrations of daunorubicin at 48 hours. Arena et al., (1972) also investigated the immediate effects of adriamycin on the heart in rabbits and dogs. Rabbits developed tachycardia and T-wave inversion, shown by electrocardiographic examination. Dogs developed a temporary increase in coronary and aortic flow and arterial blood pressure, followed by a decrease in coronary flow to well below normal. Tachycardia also occurred. Pretreatment with strophanthin apparently inhibited the appearance of toxic cardiac effects.

Benjamin, Riggs and Bachur (1973) described the disappearance of adriamycin and its metabolites from the plasma in man. An initial half-life of 1.1 hours suggested widespread and rapid uptake of adriamycin by the tissues. This was followed by a prolonged second phase of 16.7 hours. Metabolites were present in the plasma for 31.7 hours. The drug appears to undergo

extensive metabolism in the liver (Creasey, McIntosh Odujinrin, Aspnes, Murray and Marsh Brescia /1976). Metabolites produced in the liver account for 50% of the compounds excreted in urine, the remainder being the parent drug. However, only a relatively small percentage of the administered drug can be accounted for in the urine and the remainder is presumed to undergo biliary excretion. When the drug is being administered to human patients, pre-existing abnormalities of renal function seem to be of little importance but impaired liver function is known to delay metabolism of the drug and may result in severe toxicity. The long plasma half-life of adriamycin means that there is a prolonged effective drug level after a single injection. When this was discovered, medical opinion changed about dosage schedules - early work in mice had suggested that frequent dosage was more effective, but experience with the drug in clinical practice showed that toxicity was decreased and antitumour activity unaffected if an intermittent single high dosage schedule was adopted once every 3 weeks (Benjamin, Wiernick and Bachur 1974).

The main toxicity problems with adriamycin are alopecia, severe stomatitis with ulceration, myelosuppression, gastroenteritis, cardiotoxicity and chemical cellulitis if extravasation occurs during intravenous administration. It also causes sclerosis of smaller blood vessels if injected directly into them. Stomatitis appears to be much less of a problem with three-weekly dosing than with more frequent dosing, though why this is so is not clearly understood. This was first reported by Kenis, Michel

Rimoldi, Israel and Levy (1972).

Myelosuppression is the acute dose-limiting toxic effect of adriamycin, but it is rapidly reversible and is said to be platelet-sparing in humans. The nadir of leukopenia occurs about 12 days after administration and the white cell counts have returned to normal within a further 7 days. Benjamin (1975) discusses this and other complications of adriamycin therapy and how they can be dealt with.

Perhaps the most serious side effect of adriamycin is its effect on the heart. A large amount of literature exists on the subject of adriamycin cardiotoxicity. Many theories have been advanced regarding the way in which the drug damages the heart, what can be done to prevent it happening during adriamycin therapy, and methods of early detection of the damage before it is fatal.

The cardiac abnormalities produced by adriamycin in man are either (a) electrocardiograph (ECG) alterations or (b) congestive heart failure (CHF) (Cortes, Lutman, Wanka, Wang, Pickren, Wallace and Holland 1975).

(a) ECG alterations are not specific, they may occur at any time during adriamycin therapy and at any dose level. Abnormalities of rhythm, premature atrial or ventricular contractions, atrial flutter and fibrillation, decreased QRS voltage, a variety of ST-T wave abnormalities, and prolongation of QT interval have all been reported. Decreased QRS voltage is perhaps the only

abnormality which is consistently seen and this may reflect the diffuse nature of the cardiac damage.

(b) CHF occurs after prolonged dosage with adriamycin, usually occurring in patients who have received more than 550-600 mg/m². The damage to the heart appears to be cumulative and irreversible. Minow, Benjamin and Gottlieb (1975) cited two human cases whose therapy with adriamycin was discontinued and then started 6 months and 13 months later. There was no evidence of cardiac damage at the end of the initial course but fatal cardiotoxicity developed soon after starting adriamycin again. They also described a case in whom severe CHF developed three and a half months after stopping adriamycin therapy, cardiac examinations having been normal at the end of treatment. The occurrence of CHF is apparently not necessarily related to ECG changes, and it is usually sudden in onset. It responds poorly to treatment, unless mild, when forced rest, and administration of digitalis and diuretics may keep the patient alive.

Pathological examination of the heart in a case of adriamycin cardiomyopathy shows that the organ is enlarged, flabby and pale in colour. The coronary arteries and the valves are normal but the ventricles are usually dilated and may be hypertrophied.

The histopathological appearance of the myocardium reveals a decreased number of myocardial cells with

degeneration of the remaining cells, interstitial oedema and, if the patient has survived the acute phase, interstitial fibrosis. There are no indications of an inflammatory response, either acute or chronic. Examination of the tissue with the electron microscope shows in more detail, cellular loss and degeneration - in particular the mitochondria are severely damaged (Minow, et al., 1975).

Certain risk factors have been identified in human patients. Most authors are agreed that the cardiotoxic threshold is approximately 550 mg/m^2 total dose. The risk of cardiotoxicity is increased in patients who have had prior radiation therapy to the heart, who have had cyclophosphamide therapy, or who have had a history of heart disease. In these patients the maximum recommended total dose is 450 mg/m^2 body surface area.

In children, cardiotoxicity may occur even before this dose is reached. Prout, Richards, Chung, Joo and Davis (1977) cite two cases of children who developed CHF at 350 mg/m^2 and 400 mg/m^2 . Both had received prior thoracic irradiation, however.

The efficacy of adriamycin as a single agent in cases of lymphoma was considered by Carter (1975). Quoting a number of authors, he stated that the objective response rate was 37%. These were cases which were refractory to standard treatments. A reference not quoted by Carter was that of Cimo, Rudders and Hensleigh (1974). Out of 14 patients, they had an objective response in five

(36%), three with Hodgkin's and two with non-Hodgkin's lymphomas. All these responders had had previous remissions but had relapsed on conventional therapy. The median duration of response was 7 weeks. Short response duration has been reported by most authors when adriamycin is used alone in lymphoma cases. More recently, adriamycin has been used in combination with other drugs, for example cyclophosphamide, bleomycin, vincristine and prednisolone (McKelvey 12 co-authors (1976), Rodriguez, Cabanillas, Burgess, McKelvey, Valdivieso, Bodey and Freireich (1977), Skarin, Rosenthal, Moloney and Frei (1977), O'Connell, Silverstein, Kiely and White (1977)).

Using the information gained from the literature, trials were designed to investigate the effects of adriamycin in normal and lymphosarcomatous pigs.

THE EFFECTS OF ADRIAMYCIN IN NORMAL PIGS

MATERIALS AND METHODS

Ten pigs were used, eight females and two castrated males. These were litter-mates, bred from proven non-carrier parents of the lymphosarcoma strain. They were divided into three groups of two pigs in each, low-dose, medium-dose and high dose, and one group of four control pigs.

The pretreatment procedure for this experiment was as described in the general methods section. All the pigs were sensitised to DNFB at 6 weeks of age, and tested 16 days later. Weekly blood sampling was started when they were 9 weeks old. White blood cell counts, differential counts, red blood cell counts, haemoglobin concentrations, PCV, thrombocyte counts and blood urea levels were measured from the haematology sample, and serum samples were examined for levels of serum alkaline phosphatase, and serum glutamic-oxaloacetate transaminase (SGOT), although the tests on serum were not carried out every week because of the expense involved. If there was a rise in the SGOT level, total bilirubin was also measured from the serum sample. By using these measurements along with clinical observations, it was hoped to detect evidence of bone marrow, liver or cardiac toxicity. Clinical examination was started at the same time as the blood sampling and con-

sisted of daily observations on general fitness and appetite, and weekly detailed clinical examination, including recording of rectal temperature, examination of mucous membranes of the eyes and mouth, condition of skin and feet and auscultation of the thorax, with particular attention to heart sounds. Weekly weight recording was also started at 9 weeks of age.

On the day following the fourth pre-treatment blood sample, the first dose of adriamycin was given. The pigs were 12½ weeks old at this stage. The adriamycin dose levels were as follows:-

Pig No. 40)	GROUP A	- 1mg/kg
Pig No. 45)		
Pig No. 47)	GROUP B	- 2mg/kg
Pig No. 49)		
Pig No. 41)	GROUP C	- 4mg/kg
Pig No. 48)		
Pig No. 39)	GROUP D	- Saline equivalent in volume to the medium dose.
Pig No. 42)		
Pig No. 43)		
Pig No. 46)		

All the pigs were given intramuscular injection of 4% azaperone ('Suicalm', Janssen) at a rate of 4mg/kg, and were left alone for 20 minutes to allow the tranquilliser to act. This not only made the pigs easier to handle, but also dilated the superficial veins, facilitating

intravenous injection. The drug was prepared for injection by dissolving 50 mg Adriamycin powder (Pharmitalia) in 10 mls of "Water for Injection" and the calculated dose was drawn into a syringe, making sure that all air bubbles were expelled. This dilution made the preparation more concentrated than recommended on the instruction leaflet, but it was decided that there was more chance of the pig moving and causing perivascular extravasation if the volume to be injected was very large. With the pig suitably restrained, the ear was swabbed with spirit on cotton wool and injection made into an ear vein, using a 23 gauge "Miniven" set (Portex). After all the drug had been injected, 5 to 10 mls of normal saline was injected to flush out the vein, otherwise phlebosclerosis would have occurred. Saline only was injected intravenously in the controls. When the Miniven needle was removed from the vein, a piece of cotton wool was pressed on to the ear over the injection site until any bleeding stopped. The pigs were then returned to their pen and left to recover from the effects of the tranquiliser. This took several hours.

This procedure was repeated every 3 weeks for six courses. Actual doses of adriamycin in mg were as in Table 24 with total doses for each pig.

During the 18 weeks of the treatment period, clinical examination and weighing were carried out as

TABLE 24

DOSES OF ADRIAMYCIN (mg)

Group	Pig	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Total
A (1mg/kg)	40	24.5	27.0	34.0	41.0	46.0	51.5	224.0
A "	45	16.0	16.5	20.0	23.5	26.0	29.0	131.0
B (2mg/kg)	47	24.5	30.0	38.5	43.5	51.0	56.0	243.5
B "	49	37.0	40.0	47.0	53.0	58.0	63.0	298.0
C (4mg/kg)	41	76.0						76.0
C (4mg/kg)	48	88.0	82.0	88.0				258.0

before, except that if an animal was noticed to be off colour, it was given a detailed examination immediately. After the second dose of adriamycin, it was thought necessary to bleed the pigs at 9 days after dosing, as well as at 6, 13 and 20 days.

DNFB skin tests were repeated 1 week before the last dose of adriamycin.

After the fifth and sixth doses of adriamycin, it was decided that clinical examination and auscultation of the heart might be insufficient to indicate the presence and nature of any cardiac abnormalities, and therefore attempts were made to obtain electrocardiographic (ECG) recordings. In order to do this, the pigs either had to be heavily sedated or anaesthetised, because any movements made the traces unreadable. This was achieved using azaperone as before, followed by a small intravenous dose of pentobarbitone, ("Sagatal", May and Baker) to effect. The ECG leads were attached to the left fore-leg, right fore-leg and hind-leg, by means of metal needles introduced under the skin, to ensure the best possible electrical contact. Despite every precaution, there were great technical difficulties with the machine being used (Videograph 1A with ECG writer, Medical and Industrial Equipment Ltd., London) and some of the traces were completely useless. Unfortunately the pig with the most obviously abnormal heart beat died before a trace could be obtained.

Nine days after the last dose of adriamycin, two of the four control pigs and one of the group A pair were killed by intravenous injection of 20% pento-barbitone sodium. All pigs in Groups B and C had already died (See Results).

All dead pigs, whether they had been destroyed or had died suddenly, were autopsied as soon as possible.

The three pigs left alive were given no further treatment and detailed clinical examinations and weighing were stopped. Weekly blood sampling was continued for 5 weeks more, to allow any effects of the drug to disappear. These pigs were then killed and autopsied.

A final skin test was carried out 2 days before euthanasia.

RESULTS

Clinical Examinations (For clinical records see Appendix II)

Throughout the experiment, all the control pigs remained well, except that control pig number 42 was injured by the others 2 days before being destroyed at the end of the treatment period. Control pig number 39 was the runt of the litter and therefore was much smaller than the rest. It had a slight navel infection before the start of the treatment period but recovered from this rapidly.

The toxic effects of adriamycin became obvious most rapidly in the Group C pigs (4mg/kg). Pig number 41 died suddenly overnight 9 days after its first dose of adriamycin. Before it died, it was noted to have purpura, and subcutaneous haematomata, mainly on the abdomen (See Plate 6), but also on its limbs and ears. It also developed mouth ulcers.

The other Group C pig, number 48, survived three doses of adriamycin, but during the 2nd week after its third dose had to be destroyed on humane grounds. During the treatment period, its body weight remained almost unchanged (fluctuating between 20 and 22kg) but it lost condition rapidly. It had slight diarrhoea for much of the time. This became moderate in the 3rd week after the second dose, improved again and then became very severe after the third dose. It was then separated from its litter mates to prevent bullying. The pig became listless with no interest in its surroundings, its appetite decreased and it had large mouth ulcers. Its mucous

membranes were pale. With the diarrhoea, it became dehydrated though it tended to drink a great deal of water. Its skin was dry, wrinkled and scurfy, with definite hair loss, especially on the flanks, all its hooves became dark in colour and began to separate at the coronary bands. These hoof lesions were obviously infected, as there was exudation of pus when pressed gently. It shivered and its rectal temperature rose to 40.7°C. It had tachycardia (over 200/minute) and its superficial lymph nodes were enlarged. The animal could hardly stand when it was decided to destroy it and it would undoubtedly have died very shortly anyway see Plates 7 and 8.

The pigs in Group B (2mg/kg) took longer to show signs of toxicity. Both remained in good health for the first two courses of adriamycin, despite having been a little thin to start with, but during the 3rd week after the second dose, both were noted to be rather listless. Neither were off their food, however. In the 1st week after their third dose, one pig, number 49, developed a cardiac arrhythmia, which did not occur at regular intervals. The heart sounds themselves were normal. The following week the heart sounds were normal and regular. In the 2nd week after the fourth dose, this abnormality was noted again on auscultation and it became very pronounced. Both pigs were by this time noticeably thinner than the control and Group A pigs and had patchy alopecia, again particularly

on the flanks. During the 1st week after the fifth dose of adriamycin, the heart rates of both pigs were irregular, and in 49 extra heart sounds were heard.

Attempts were made to obtain ECG tracings from these pigs but due to technical difficulties with the machine, only one rather poor trace was obtained from Lead III on pig number 47. The intervals between QRS complexes, measured in millimetres on the trace, were found to vary from 14 to 20, in contrast to the intervals in traces from the control pigs which varied a little between pigs but were reasonably constant for any one animal.

In order to ascertain whether or not there were other abnormalities present, it would have been necessary to compare the traces with a pretreatment trace from the same animal. It was possible to obtain another trace from pig 47, although it was very ill by this time with a definitely abnormal rhythm to the heart sounds, after the sixth dose of adriamycin, there was a marked difference between the traces. The heart rate was very rapid and on the same lead, Lead III, the appearance and heights of the T-waves had changed, also the heights of the QRS complexes were 10 to 12mm, compared with 13 to 16mm in the previous trace. Heights of QRS complexes in the control animals on Lead III were variable, but constant for each animal. Pig number 49 died before the ECG machine was repaired and no traces

were obtained. Since its heart sounds were much more irregular than those of 47, this was unfortunate. Before 47 died, it developed severe haemorrhagic diarrhoea. 49 had buccal ulceration, mainly inside the lower lips and on the gums round the base of the incisor teeth. Body weight gains were obviously affected in this group (See Table 25)

Both pigs in Group A (1mg/kg) remained fit with excellent appetites. One pig, 45, developed a large ulcer between the gum and lower lip below the incisor teeth in the third week after the fourth dose. This healed rapidly, but another ulcer developed in the same position after the sixth dose. ECG tracings were taken the day before half of the pigs were destroyed at the end of treatment. These did not reveal any abnormality or alteration in rhythm in either Group A pig, although a slight irregularity in rhythm had been noted in pig 45 after the fifth dose of adriamycin. Body weight gains in this group varied (See Table 25).

In no pig, other than in the highest dose group, was there any evidence of fever. Rectal temperatures stayed below 40°C, usually between 38 and 39°C. This seemed to be the normal range for pigs of post-weaning age in the piggery.

DNFB Skin-Test Results

It can be seen from Table 26, that all the treated pigs which survived to be tested, showed a decrease in reaction score by the time of the second test. However,

TABLE 25

BODY WEIGHT GAINS - ADRIAMYCIN: NORMAL PIGS

Pig No.	Dose Level	Wt. at start of treatment (kg)	Wt. at end of 15 weeks treatment or death* (kg)	Gain (kg) or loss
41	4 mg/kg	19.00	20.00 * (1½ weeks)	+1.00
48	4 mg/kg	22.00	20.00 * (8 weeks)	-2.00
47	2 mg/kg	12.25	28.00	+15.75
49	2 mg/kg	18.50	31.50	+13.00
40	1 mg/kg	24.50	51.50	+27.00
45	1 mg/kg	15.75	39.00	+13.25
39	Control	8.50	27.00	+18.50
42	Control	18.50	42.75	+24.25
43	Control	22.50	53.00	+30.50
46	Control	21.75	48.00	+26.25

TABLE 26

DNFB SKIN - TEST - RESULTS ADRIAMYCIN: NORMAL PIGS

Groups	Pig Number	At sensi- tisation	Pre-treat- ment	During 1	treatment 2	After treat- ment
D	39	3	4	4	1	3
(Control)	42	3	4	1	3	Dead
	43	3	4	3	2	Dead
	46	3	4	2	4	1
A	40	3	4	3	2	2
(low dose)	45	3	4	2	0	Dead
B	47	3	2	4	1	Dead
(med.dose)	49	3	3	4	2	Dead
C	41	3	4	Dead	-	-
(high dose)	48	3	4	Dead	-	-

the results are confused by the poor second test scores of control pigs 39 and 43.

Haematology

The effects of adriamycin on white blood cells were shown most obviously by the counts obtained from the two pigs given the highest dose, (4mg/kg). Because these animals died fairly early in the experiment, their counts are not included in the table of group means but are to be found with the figures for individual pigs (Appendix II). There was a marked fall in total white blood cell counts by 6 days after dosing in both pigs. No. 41 was leucopenic (5.5×10^9 /litre) just before it died 9 days after the first dose. In pig no. 48, the counts improved following the nadir after the first two doses, but 6 days after the third dose, the count fell to a nadir of 3.9×10^9 /litre and was still low at 13 days (6.7×10^9 /litre). It was destroyed 3 days later in extremis.

Figure 11 illustrates the absolute lymphocyte and neutrophil counts of these pigs compared with the mean lymphocyte and neutrophil counts of the four saline-treated controls. From these graphs, it can be seen that there were effects on both lymphocytes and neutrophils, but the effect on the lymphocyte counts was more dramatic and prolonged, recovery taking place between 13 and 20 days after each treatment in pig no. 48. It is interesting that neutrophil counts were beginning to recover in both pigs before death but

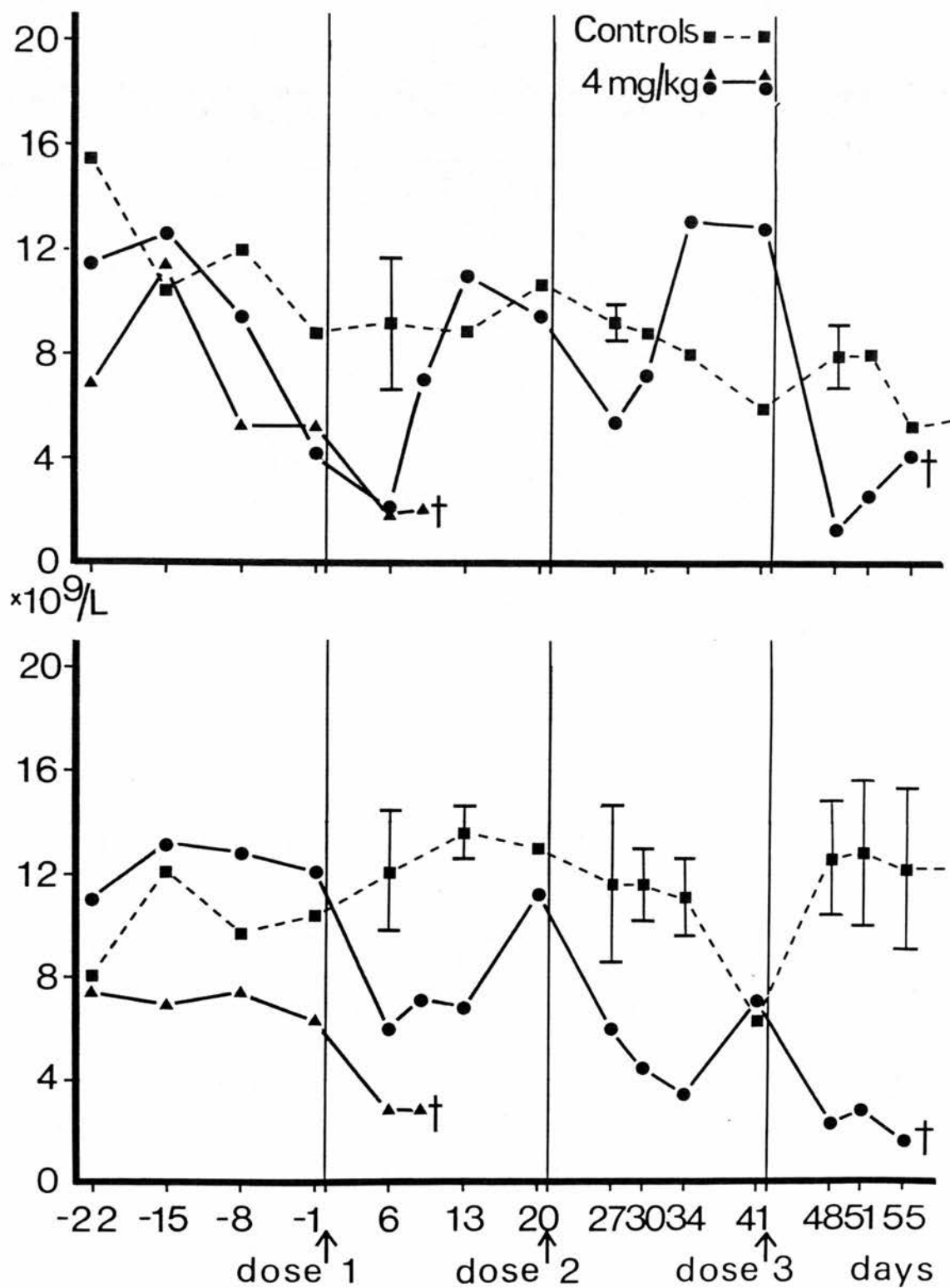


FIGURE 11

Absolute neutrophil counts (above) and lymphocyte counts (below) of two normal pigs treated with 4mg/kg adriamycin compared with mean counts of four saline-treated controls.

lymphocytes were not. Monocyte counts also appeared to be depressed in the treated pigs, but since monocyte counts are usually low and very variable, it is difficult to draw definite conclusions.

The total white cell counts and absolute neutrophil, lymphocyte and monocyte counts (means \pm S.D.) of Groups A, B and D are shown in Table 27. There was a marked effect on mean total white cell counts from 6 to 9 days after dosing in Group B when compared with Group D as illustrated in Figure 12. Mean counts in Group A were also lower than Group D, though this was less marked and only from the third dose onwards. Standard deviations were larger in Group A than in Group B, and neither pig in Group A was ever leucopenic.

When the mean neutrophil counts at 6 days after dosing are examined (Table 28A) the standard deviations are wide. There were no consistent or dose-related effects, though the counts of the treated pigs did tend to be lower than those of the controls.

However at 6 days after each dose, the mean lymphocyte counts of Group B were consistently lower than those of Group A, which in turn were consistently lower than the controls, indicating a clear-cut dose-related effect. (Table 28B)

There were no differences in mean monocyte counts between Groups A, B and D, which could be attributed to the treatment.

Eosinophils and basophils were noted when seen in blood smears but because of the small numbers it is

TABLE 27

ADRIAMYCIN : NORMAL PIGS											
GROUP D - SALINE CONTROLS				GROUP A - 1 mg/kg ADRIAMYCIN				GROUP B - 2mg/kg ADRIAMYCIN			
WBC - MEANS \pm 1 S.D.				T.NEUTROPHILS - MEANS \pm 1 S.D.				LYMPHOCYTES - MEANS \pm 1 S.D.			
GROUP D				GROUP A				GROUP B			
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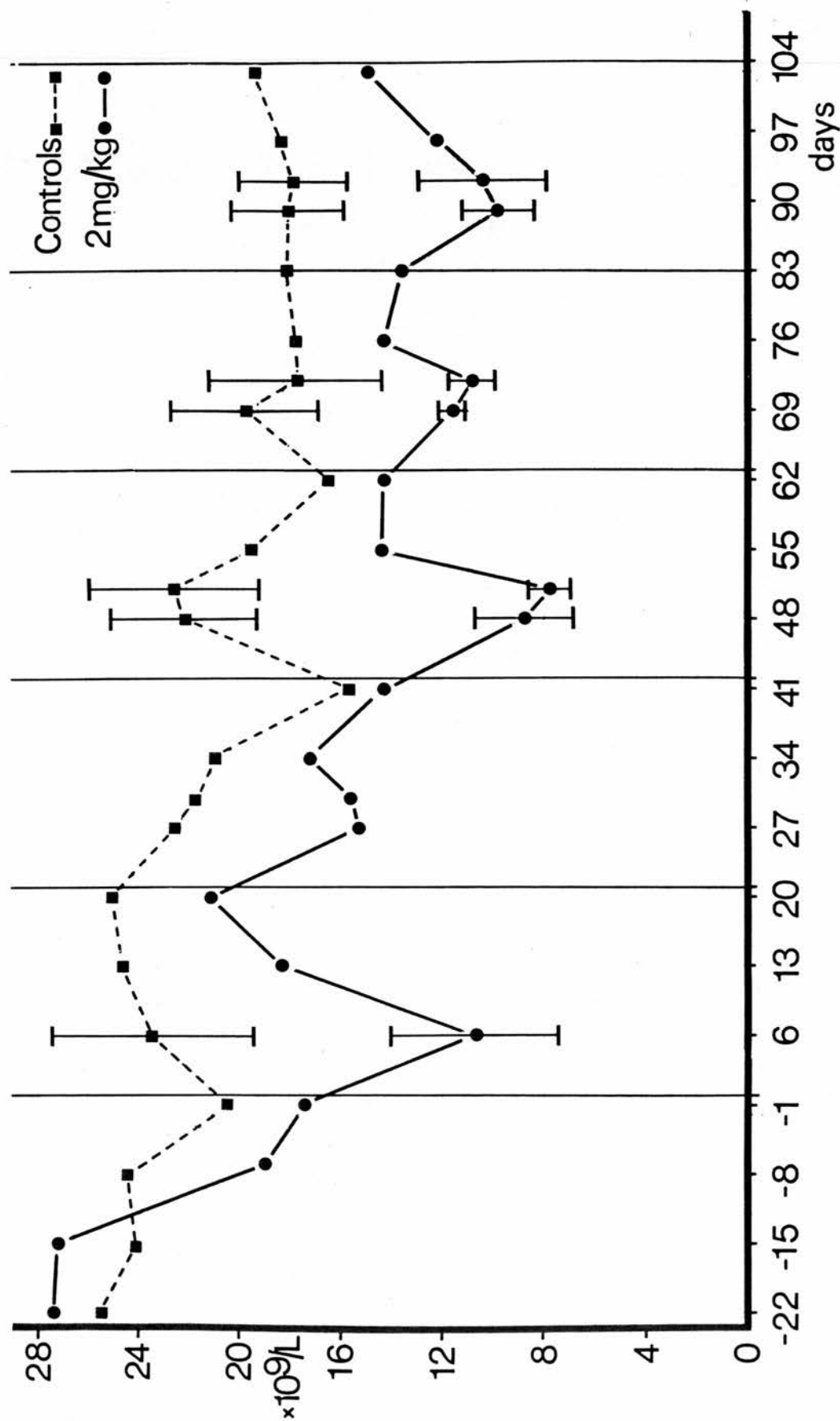


FIGURE 12

Mean WBC counts of two normal pigs treated with 2mg/kg adriamycin compared with mean counts of four saline-treated controls. Vertical lines indicate times of dosing.

ADRIAMYCIN IN NORMAL PIGS DOSED EVERY THREE WEEKS

TABLE 28A

Mean neutrophil counts \pm 1 S.D.

	Control Group D	Group A (1mg/kg)	Group B (2mg/kg)
Last Bleed Before Dosing	8.8 \pm 2.1	10.6 \pm 3.9	5.8 \pm 2.5
Six Days After Dose 1	9.2 \pm 2.5	7.5 \pm 5.5	2.1 \pm 1.4
Six Days After Dose 2	9.3 \pm 0.7	9.5 \pm 4.0	8.6 \pm 3.6
Six Days After Dose 3	8.0 \pm 1.2	4.9 \pm 0.5	4.6 \pm 0.9
Six Days After Dose 4	8.2 \pm 1.9	5.7 \pm 0.6	6.3 \pm 2.0
Six Days After Dose 5	6.3 \pm 1.2	4.9 \pm 1.1	5.5 \pm 2.1
Six Days After Dose 6	4.9 \pm 2.1	4.4 \pm 2.1	—

TABLE 28B

Mean lymphocyte counts \pm 1 S.D.

	GROUP D	GROUP A	GROUP B
Last Bleed Before Dosing	10.4 \pm 3.0	10.4 \pm 1.4	10.0 \pm 5.1
Six Days After Dose 1	12.1 \pm 2.3	10.7 \pm 3.6	7.1 \pm 3.7
Six Days After Dose 2	11.6 \pm 3.0	9.3 \pm 2.7	5.5 \pm 1.0
Six Days After Dose 3	12.6 \pm 2.2	6.3 \pm 2.6	3.4 \pm 0.9
Six Days After Dose 4	10.5 \pm 1.0	7.2 \pm 2.4	4.6 \pm 2.2
Six Days After Dose 5	10.5 \pm 2.3	7.0 \pm 2.1	3.6 \pm 0.6
Six Days After Dose 6	6.9 \pm 2.1	4.9 \pm 1.7	—

not possible to say whether they were affected by the treatment or not.

As with the white cells, effects on the red blood cell picture were most readily seen in the results from pig 48 which survived three doses of 4 mg/kg adriamycin. (See Appendix II) Figure 13 shows the depression in Hb, PCV and red cell counts which occurred in this animal at 13 days after each dose, compared with mean values from the four control pigs (one standard deviation shown at 13, 34 and 55 days, for others see Table 29). Red blood cell (RBC) counts remained slightly below those of the controls all through the treatment period, but were beginning to recover from the nadir by 20 days after the first and second doses. The highest values were obtained 6 days after the following dose, before its effect became apparent. This also happened with Hb concentration and PCV. In fact, the "rebound" values for Hb and PCV after the second dose were even higher than the mean values for the controls.

The mean corpuscular volume (MCV) began to increase after 34 days of the treatment period and the mean corpuscular haemoglobin (MCH) also showed a slight increase after this time (See Appendix II). Mean corpuscular haemoglobin concentration (MCHC) values were similar to those of the controls.

Pig 41 which died after one dose of 4 mg/kg showed no effect on the red cell picture at 6 days after dosing,

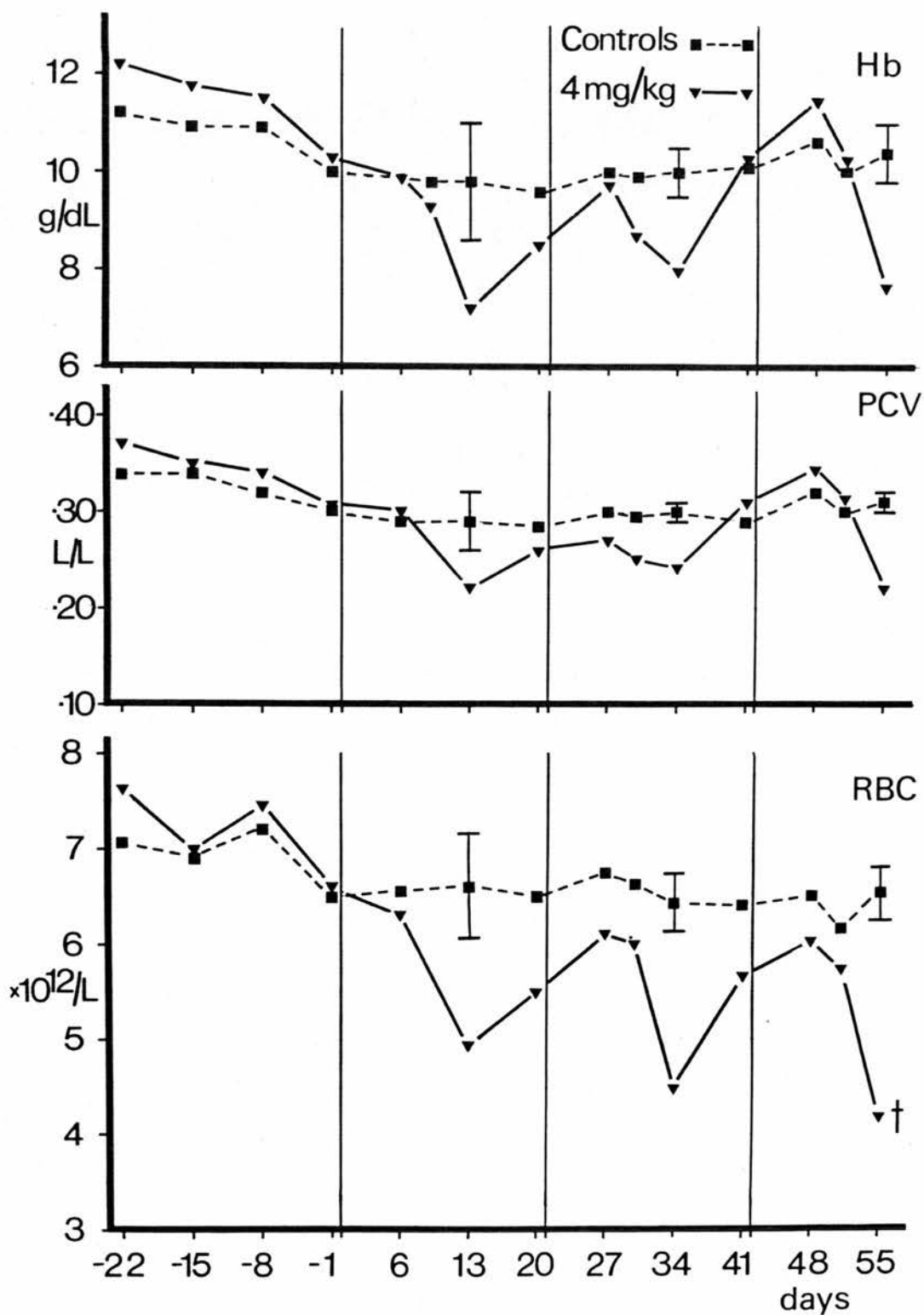


FIGURE 13

Hb (above), PCV (centre) and RBC counts (below) of one normal pig treated with 4mg/kg adriamycin, compared with mean values of four saline-treated controls. Vertical lines indicate times of dosing.

as with 48. The Hb concentration was 10.9 g/dl. At 9 days, only the Hb concentration was measured, the low figure of 6.1 g/dl being obtained. This was not unexpected considering the multiple haemorrhages which had occurred in this animal.

The mean RBC counts, Hb and PCV values for the pigs in Group B showed a milder effect, in that they were lower than those of the controls throughout the treatment period, and especially at 9 and 13 days after the third, fourth and fifth doses. The lowest figures for this group were obtained on day 55, after which there was a rise in the mean values. RBC counts in the control groups were also low at this time (See Table 29).

Mean values for RBC counts of pigs in Group A also tended to be lower than those of the controls during treatment, but taking into account the standard deviations which overlap, it is unlikely that there was any real effect. Mean PCV and Hb levels were actually higher than those of the controls on many occasions.

There was no consistent effect on MCV, MCHC or MCH in either Group A or B, the mean values being similar to those of the controls.

The effects of adriamycin on thrombocyte counts were again shown, quite dramatically, by the results from the two high-dose pigs 41 and 48. / ^{See Figure 14.} After each dose, there was a severe depression of thrombocyte counts, the lowest

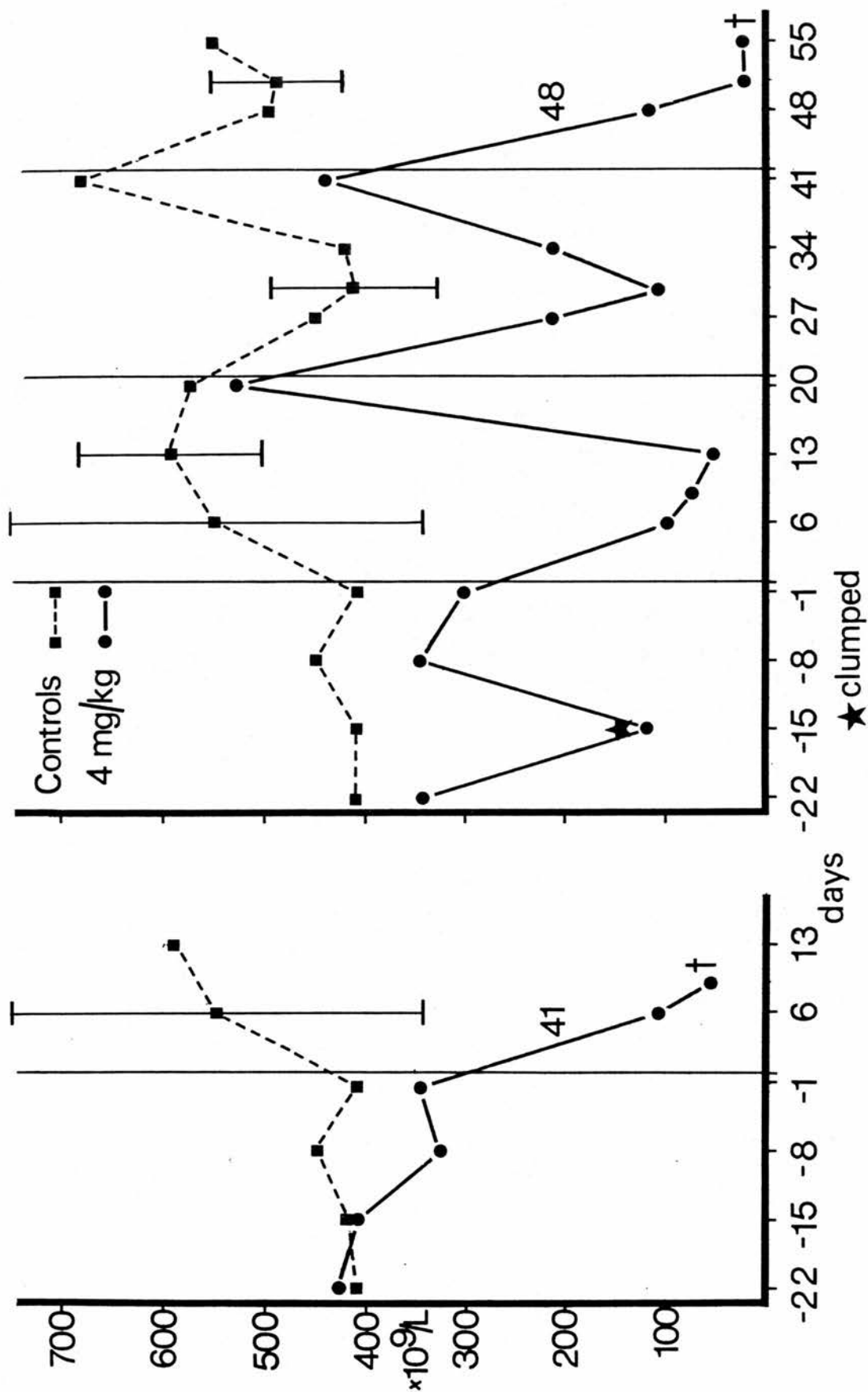


FIGURE 14

Thrombocyte counts of two normal pigs treated with 4mg/kg adriamycin compared with mean counts of four saline-treated controls. Vertical lines indicate times of dosing.

figures being obtained at 9 and 13 days after dosing.

Pig 48 recovered remarkably well from the thrombocytopenia on two occasions, the counts at 20 days being similar to those of the controls. However, after the third dose, the count fell to 22×10^9 /litre.

The pigs in Group B showed a milder depression in mean thrombocyte counts compared with the controls, especially after the third, fourth and fifth doses (See Figure 15 and Table 30. Standard deviations are shown on the graph only where there is likely to be an important difference). The nadir was around the 9th day. Neither of the Group B pigs was ever dangerously thrombocytopenic.

There was no effect on thrombocyte counts in Group A which could be attributed to the treatment.

Serum Enzymes

Alkaline Phosphatase

At 9 weeks of age, when blood sampling started, the serum alkaline phosphatase levels in all ten pigs ranged from 93 to 204 IU/litre. These were within the normal range for 9 week old pigs (Imlah, 1968). During the following 3 weeks of the pretreatment period, there was considerable fluctuation in the levels of alkaline phosphatase, and mean levels for the low and medium dose group pigs were lower than those for the control and high dose pigs. During the treatment period, for the 11 weeks during which alkaline phosphatase levels were measured, the figures for Group A and B pigs continued to

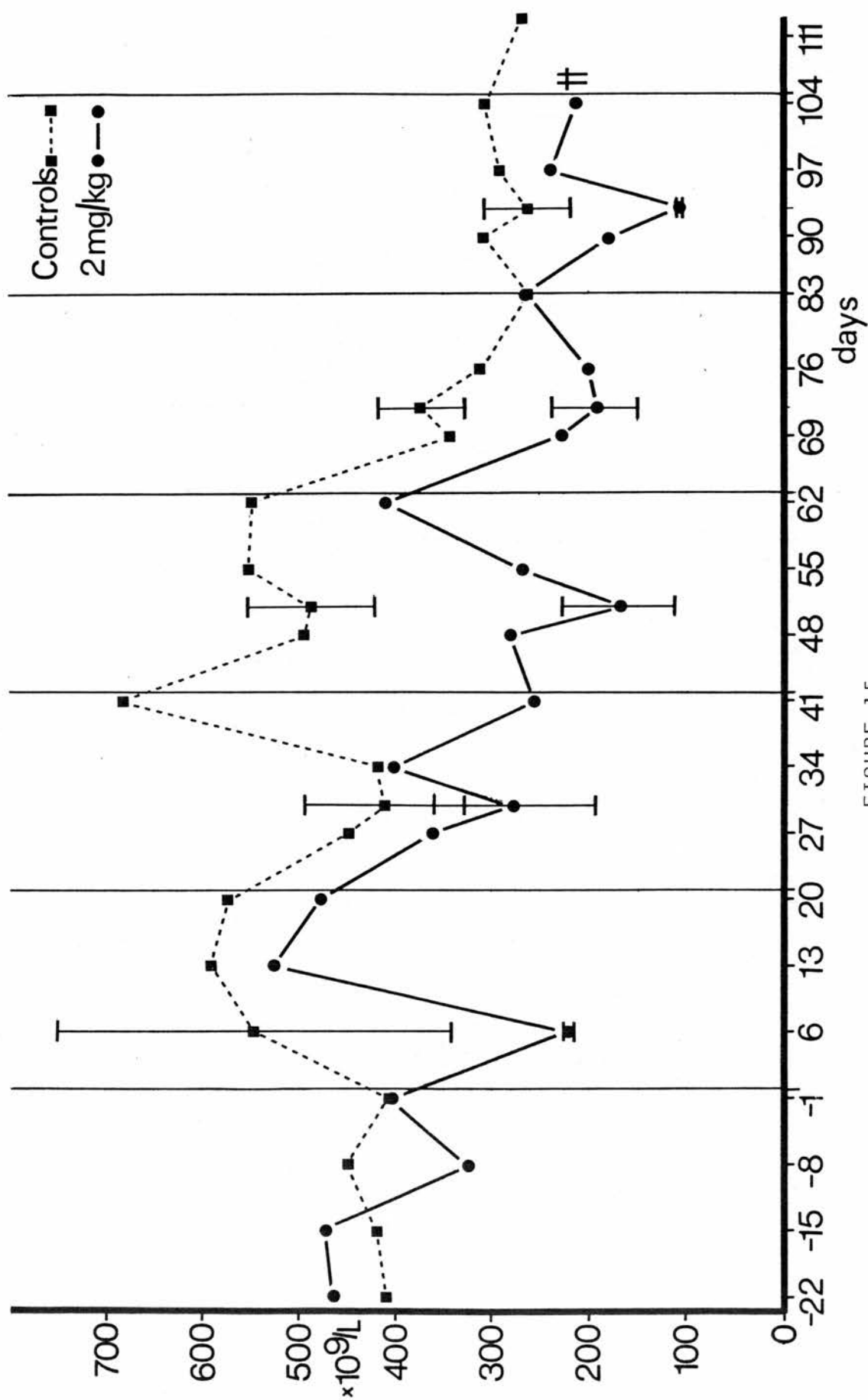


FIGURE 15

Mean thrombocyte counts of two normal pigs treated with 2mg/kg adriamycin compared with mean counts of four saline-treated controls. Vertical lines indicate times of dosing.

TABLE 30

ADRIAMYCIN : NORMAL PIGS - MEAN THROMBOCYTE COUNTS \pm 1 S.D.
IN CONTROL, LOW AND MEDIUM DOSE GROUPS DURING
THE TREATMENT PERIOD

DAYS FROM START OF DOSING	GROUP D (Controls)	GROUP A (1 mg/kg)	GROUP B (2 mg/kg)
- 22	410 \pm 17	473 \pm 44	465 \pm 10
- 15	418 \pm 73	403 \pm 7	473 \pm 13
- 8	448 \pm 37	512 \pm 118	325 \pm 163
- 1	408 \pm 49	371 \pm 14	417 \pm 85
Dose 1 - 0			
+ 6	546 \pm 204	314 \pm 22	221 \pm 5
13	592 \pm 89	711 \pm 56	528 \pm 78
20	572 \pm 177	477 \pm 33	478 \pm 33
Dose 2 - 21			
27	449 \pm 64	554 \pm 46	362 \pm 71
30	412 \pm 81	433	277 \pm 83
34	419 \pm 44	468 \pm 88	402 \pm 64
41	680 \pm 263	578 \pm 263	257 \pm 196
Dose 3 - 42			
48	494 \pm 54	431 \pm 40	281 \pm 12
51	487 \pm 66	427 \pm 15	169 \pm 57
55	552 \pm 143	541 \pm 18	267 \pm 7
62	448 \pm 71	440 \pm 55	409 \pm 51
Dose 4 - 63			
69	343 \pm 65	304 \pm 16	227 \pm 33
72	372 \pm 45	268 \pm 34	193 \pm 43
76	312 \pm 40	331 \pm 41	201 \pm 0
84	262 \pm 36	294 \pm 12	266 \pm 103
Dose 5 - 84			
90	309 \pm 36	293 \pm 18	181 \pm 2
93	262 \pm 45	264 \pm 36	109 \pm 4
97	290 \pm 84	269 \pm 33	241 \pm 60
104	307 \pm 78	276 \pm 1	213 \pm 15
Dose 6 - 105			
111	267 \pm 41	214 \pm 23	-

TABLE 31

ADRIAMYCIN : NORMAL PIGS

MEAN LEVELS OF SERUM ALKALINE PHOSPHATES \pm ISD (i.u./litre)

DAYS FROM START OF DOSING	GROUP D - CONTROLS	GROUP A - 1 mg/kg	GROUP B - 2 mg/kg
- 22	146 \pm 48	148 \pm 12	120 \pm 0
- 15	132 \pm 20	111 \pm 13	77 \pm 50
- 8	96 \pm 36	77 \pm 16	88 \pm 14
- 1	132 \pm 23	100 \pm 16	109 \pm 3
Dose 1 - 0			
6	104 \pm 20	85 \pm 4	67 \pm 3
13	138 \pm 20	70 \pm 33	82 \pm 29
20	108 \pm 27	87 \pm 8	96 \pm 13
Dose 2 - 21			
27	123 \pm 24	104 \pm 16	106 \pm 35
30	134 \pm 25	93 \pm 38	98 \pm 15
34	134 \pm 12	88 \pm 14	99 \pm 15
41	117 \pm 23	89 \pm 16	101 \pm 44
Dose 3 - 42			
48	137 \pm 16	99 \pm 16	71 \pm 16
51	112 \pm 31	112 \pm 6	53 \pm 11
55	138 \pm 34	115 \pm 18	80 \pm 11
62	132 \pm 23	107 \pm 6	125 \pm 7
Dose 4 - 63			
69	93 \pm 20	62 \pm 1	88 \pm 11
72	114 \pm 22	66 \pm 9	114 \pm 34
76	Discontinued		

be lower than those of the controls, particularly at 6 and 9 days after the first and third doses of adriamycin. However, the effect was not consistent or dose-related, and since the fluctuations seemed to occur in a similar pattern to those recorded in the control animals, it is unlikely that this was due to the action of the drug.

If the serum alkaline phosphatase levels of the high-dose animals are individually compared with the mean \pm S.D. of the control animals, it can be seen that, at 6 days after the first dose of adriamycin, the pig which died at 9 days had an alkaline phosphatase concentration which was higher than those of the controls, but which was almost exactly the same as that recorded in the same animal before it was dosed. However, the surviving high-dose pig showed a decrease from 136 IU/litre before dosing, to 34 IU/litre 6 days after dosing, and the concentration remained low thereafter, until its death.

See Table 31 and Table 33 (Appendix II)

SGOT

Levels of SGOT in all ten pigs were low at the start of blood sampling, ranging from 16.5 to 25.2 IU/litre. From time to time during the experiment small rises occurred in every animal, even the controls. As this enzyme indicates cell damage in liver, heart and muscle, the importance of these changes is very doubtful.

(See Table 34 in Appendix II). However, on one particular day 6 days after the first dose of adriamycin, all pigs, except one control and one high-dose animal, had elevated SGOT levels. One of the pigs given the lowest dose, number 40, had a moderately high concentration of 238 IU/litre. Total bilirubin was measured in those pigs which had SGOT levels greater than 50 IU/litre on this occasion and the following figures were obtained:-

	SGOT IU/l	Total Bilirubin $\mu\text{mol/l}$
Control	55.6	6.84
Control	124.1	6.82
Low Dose	238.0	5.47
Low Dose	148.7	6.84
Medium Dose	67.4	5.47
High Dose	147.2	2.73

None of these values for total bilirubin is greatly different from the figure of 5.8 $\mu\text{mol/litre}$ given by Baetz and Mengeling (1971) for normal swine. Since the elevation of SGOT was present in the control pigs as well as the treated animals it is unlikely that this effect was connected with adriamycin dosage.

Blood Urea (See Tables 21 to 30 Appendix II).

Blood urea measurements in the treated pigs showed no differences from those in the control pigs which could be attributed to the effects of the drug. All measurements before, during and after treatment were within the

normal range, apart from that obtained in the control pig, number 42, in the last week of treatment (11.11) which could have been associated with the injuries it received from its litter mates.

Post-Mortem Findings

Control Pigs

39.

This small, moderately well nourished pig had extensive fibrous adhesions of the lungs to the ribs and diaphragm, though the lungs themselves were normally aerated. Adhesions were also present in the pericardial sac but the heart appeared normal in thickness and the valves were normal.

There were extensive fibrous adhesions between the liver and diaphragm. There was fibrosis on the visceral surface of the liver but no adhesions to any of the other viscera.

42

This was a normal carcase but showing extensive red graze marks all over the skin, especially on the head and shoulders.

43 and 46

Neither of these carcasses showed any abnormality.

Low-Dose Pigs

40

This pig showed no abnormalities. Most lymph nodes had 3 to 4 mm of white nodular cortex but

no cortex was visible in the bronchial nodes and little in the gastric nodes.

45

This was a small, moderately nourished carcase. The only abnormality seen was approximately 20 ml of fluid in the pericardial sac. All lymph nodes had about 3 mm of white cortex visible.

Medium-Dose Pigs

47

This was a moderately developed carcase but with less fat than normal at all sites.

There was marked oedema of the eye-lids and the skin showed red blotchy mottling.

The meninges of the brain were slightly congested with some fibrin.

The thymus lobules were very small and thin.

The chest showed extensive fibrous adhesions between the lungs and the ribs, especially anteriorly, and between the diaphragmatic lobes and the diaphragm. The lungs were moderately congested and oedematous and not fully expanded.

The heart showed marked fibrous adhesions obliterating the pericardial space. The right ventricle was dilated and 4 mm thick. The left ventricle was 20 mm thick. The endocardium and cut surface showed pink/red mottling (as 49). All valves were normal except the left A-V valve which was slightly thickened, nodular and oedematous.

The abdominal cavity contained about 200 ml of cloudy brown fluid but with no fibrin or thickening of the peritoneum.

The liver was rather small with extensive fibrous adhesions between the diaphragm and the liver.

The spleen was contracted.

The cardiac region of the stomach showed blurred 1mm petechiae. There was haemorrhage in the submucosa of the fundus. The pylorus was slightly blood stained.

There was marked oedema of the mesentery and between the coils of the colon. The caecum and colon were distended with dark brown mealy fluid and the mucosa was bright red with a few small yellow foci.

The bone marrow of the femur was red and active.

All the carcase lymph nodes showed a white network in which there were small areas of red cellular tissue and no typical normal cortex.

49

This was a well-developed moderately nourished carcase. The skin over the lower limbs showed a few red ulcers and brown scabs.

There was no fluid in the pericardial sac, thorax or abdomen.

The thymus was thin. All lobes of the lungs showed slight partial collapse.

The heart showed fibrous adhesions over a 1cm area near the apex of the left ventricle. Epi-and endocardial surfaces showed "thrush-breast mottling" and also the cut surface, probably due to fatty change. The right ventricle was 4mm thick, the left 20mm, with rather flabby muscle, the valves were normal.

The liver was rather small, sharp bordered, with some fibrous adhesions to the membranous portion of the diaphragm.

The spleen was contracted.

The cardiac region of the stomach showed multiple 2mm petechiae.

There was haemorrhagic material in the submucosa of the fundus.

The serosa of the small intestine was red to dark red. Peyer's patches were visible but without obvious lymphoid nodules. The wall was not thickened.

The ileocaecal valve showed a very congested mucosa with a thin grey necrotic surface. The caecum was full of thin, fibrous, fluid food and the mucosa showed some petechiae. The coils of the colon showed multiple 2 mm yellow raised nodules surrounded by 1-2 mm wide congested zones.

The feet showed cracks between the sole and the wall at the junction between sole and bulb / ^{of the heel.} Some cracks extended down into the underlying connective tissue.

The cut surface of all lymph nodes showed little evidence of white lymphoid tissue. All showed congestion and sometimes haemorrhage.

High-dose pigs

41

This was an anatomically normal carcass with blue slightly raised haemorrhages 2-3cm diameter on the

belly and 1-2cm (not raised) on the ears and lower limbs.

On cut surface there were subcutaneous haemorrhages on the ventral abdomen and chest up to 1cm thick. The animal was not dehydrated. There was a moderate amount of blood in cut vessels.

There were multiple 1-2mm petechiae in the subcutaneous fat, especially on the sides of chest and belly.

The upper gum of the mouth showed a 2mm red ulcer opposite the lower canines.

All lymph nodes showed up prominently because they were dark red. On cut surface they were mainly composed of red haemorrhage with a 1-2mm white nodular cortex centrally. The prescapular was all red, the mesenterics showed a brown, quite extensive medulla.

The cervical thymus was very thin with small brown nodules.

The thoracic thymus was only 3mm thick, surrounded by yellow oedema.

The lungs, on the right side, showed congestion and oedema and petechiation. The left side was emphysematous with no petechia and little oedema.

Serosanguinous fluid was present in the pericardial sac, thoracic and abdominal cavities but no fibrin was present.

The heart showed many sub-epicardial petechiae and a few sub-endocardial ones. The muscle was normal.

The liver was slightly pale, lobulation not distinct. The spleen was contracted.

There were petechiae in the bladder and serosa of the stomach.

The large intestinal wall was slightly thickened with oedema between the coils of the colon. Just below the caeco-colic junction for $1\frac{1}{2}$ coils were areas of yellow/green necrosis doubling the thickness of the gut and giving it a corrugated appearance.

Cultural examination of large intestinal contents proved negative for the presence of accepted bacterial pathogens.

48

The pig was not markedly thin. The skin surface showed many 5-10mm black/brown scabs - largest on the ears and lower limbs and over the carpal region. If pulled off these left a white scab.

The blood was thin and watery.

There were a few 1-2mm depressed shallow brown ulcers on the inside of the lips and sides of the tongue.

There was no subcutaneous lesions on skinning. The thymus was thin.

Both ventricles of the heart were rather thin walled otherwise N.A.D.

The spleen was contracted.

The caecum and colon were much dilated with thin brown-grey porridge-like fluid. The wall was not thickened. The mucosa was slightly congested and finely nodular, grey in colour - possibly necrotic.

The meninges of the brain were congested and some fibrin

was present.

Limb joints showed a slight excess of clear fluid but synovial membranes and joint surfaces were normal.

Most lymph nodes showed an ill-defined cortex forming white nodules more than three quarters of the node with a pale brown medulla.

The feet showed fluctuating swellings on the lateral aspect of the bulbs of the heels. On sawing through the digits, small irregular penetrating wounds were found with variable-sized abscess cavities containing yellow pus and necrotic material, mainly under-running the sole but sometimes arising from the coronary band. Neither the joints nor the bones of the foot were involved.

The bone marrow of the femur was red and cellular.

Lymph Node and Organ Weights

Relative organ and lymph node weights in cg/kg are shown, for all treated and control pigs, in Table 32. The weights from both animals in the low-dose group are similar to those of the control group.

However the medium and high-dose animals had relative lymph node weights which were markedly greater than those of the controls. This was true for all the nodes, in both splanchnic and superficial groups. Liver, kidneys and adrenals were also enlarged, especially in the high-dose animals. In contrast, thymus and spleen

TABLE 32

ADRIAMYCIN : NORMAL PIGS		RELATIVE WEIGHTS OF LYMPH NODES AND ORGANS (cg/kg body weight)									
DOSE	PIG NUMBER	CONTROL	(SALINE)	LOW (mg/kg)	MEDIUM (2mg/kg)	HIGH (4 mg/kg)					
	42	43	39	46	40	45	47	49	41	48	
Age at death (days)	201	201	250	250	250	201	198	197	98	146	
Body Weight (kg)	18.5	22.5	8.5	21.8	24.5	15.8	12.3	18.5	19.0	22.0	
Pre Treatment	52.0	64.0	32.0	57.5	60.5	36.0	22.3	30.5	20.1	20.2	
End of Treatment	-	-	42.0	74.5	74.0	-	-	-	-	-	
End of Experiment											
Lymph Nodes	206	300	321	268	257	242	460	383	473	496	
Mesenteric and Colonic	31	34	30	18	17	30	45	49	42	55	
Gastric and Splenic	12	9	10	8	10	11	13	15	27	25	
Bronchial Group	249	343	361	294	284	283	518	447	542	576	
Total Splanchnic	39	33	35	30	27	36	60	42	47	63	
Head Group	16	8	9	11	9	15	16	21	16	23	
Iliac Group	17	4	7	5	5	8	4	7	11	13	
Prescapular	5	5	3	2	2	6	6	5	6	8	
Precrural	18	11	13	9	9	17	20	43	19	29	
Inguinal	3	1	NF	1	NF	2	3	1	NF	6	
Popliteal	98	62	67	58	52	84	109	119	99	142	
Total Superficial	81	111	134	93	106	55	34	12	40	12	
Thymus	337	250	381	430	311	250	82	126	95	107	
Spleen	2539	2780	2714	2389	2581	3028	2831	3398	3532	4335	
Liver	202	172	155	184	169	181	250	315	249	402	
Kidney (mean)	7	7	7	6	5	5	11	14	9	16	
Adrenal (mean)											

relative weights were markedly reduced in the medium and high dose groups.

DISCUSSION

It is clear from the results of this experiment that adriamycin, when administered at a dose level of 2 mg/kg or greater, produces severe toxic effects, and that these effects are cumulative.

The effects noted clinically were dullness, loss of condition, enlargement of superficial lymph nodes, buccal ulceration, patchy alopecia, diarrhoea, haemorrhage, lameness, pale mucous membranes, tachycardia and cardiac arrhythmia, fever in one animal probably as a result of infection of the feet, and possibly depression of the skin hypersensitivity response.

The haematology results indicated bone marrow depression with temporary decreases in lymphocyte, neutrophil, red blood cell and thrombocyte counts, PCV and Hb, and increases in MCV and MCH.

These effects were similar to those described in humans (Benjamin, 1975), apart from the time of the nadir in the WBC count, which was at 6 days after dosing compared with 12 days in man, and the depression of thrombocyte counts, which is not a frequent side-effect in man. Benjamin stated that tumour infiltration of the bone marrow was one of the risk factors associated with increased adriamycin toxicity, especially with regard to thrombocytopenia, therefore this was an important consideration when deciding on a dose level to be used in treating the lymphosarcomatous pigs.

Though the fall in serum alkaline phosphatase level in high-dose pig 48 is striking, it would appear to be unrelated to the adriamycin administration, unless in some way it reduced the formation of alkaline phosphatase. This enzyme is mainly produced extrahepatically, e.g. in bone, and according to Bodansky (1961), elevation of serum alkaline phosphatase in obstruction of the bile ducts and hepatitis is due to failure of the liver to metabolise and excrete the enzyme.

In the veterinary literature only four reports have been found which described trials using adriamycin in normal animals of the domestic species.

Smith and Kirk (1976) injected adriamycin intravenously at weekly intervals into seven dogs, six of which were clinically normal, and one of which had a mammary tumour. Varying dose levels were used. Generalised alopecia and stomatitis did not occur. Effects on lymphoid tissue, bone marrow and blood counts were reported. A dose level of 0.5 mg/kg produced only slight bone marrow depression, whereas the highest dose level, 2.5 mg/kg produced complete depression of haematopoietic activity. WBC counts decreased in all but one dog, both lymphoid and myeloid cells being affected. RBC counts were not measured and Hb and PCV values did not change appreciably during treatment. Total protein did not alter either. There was no mention of thrombocyte counts. All the dogs were destroyed without

allowing them to recover from the effects of treatment. Histological changes in spleens, lymph nodes, lungs, livers, bone marrow, testes and ovaries were investigated, but weights of organs or lymph nodes were not recorded.

Hennes, Theilen and Lewis (1977) administered adriamycin intravenously to normal cats, in three groups of two. Antihistamine and glucocorticoid injections were also given to minimise toxic effects. Another dose of adriamycin was given 3 weeks later. Decreases in WBC counts and diffuse alopecia with delayed hair growth were reported.

Van Vleet, Greenwood and Ferrans (1979) administered adriamycin intravenously to normal pigs at varying dose levels from 0.64 mg/kg/week to 6.4 mg/kg/week. In three out of the five dose groups, the drug was continued until all the pigs were dead, and the pigs left alive in the other groups were destroyed at the end of treatment. Survival time varied from 10 days in the highest dose group to 134 days in a low dose group, and was prolonged in younger pigs i.e. those that were 4 weeks old, and in those given smaller, more frequent doses.

The detailed results described in this paper were almost entirely pathological, though it did mention that alimentary tract lesions were manifested clinically by inappetence associated with painful oral ulcers and diarrhoea, and also mentioned marked cellulitis and necrosis of skin, muscle and ear cartilage caused by perivenous extravasation. It stated in the "Methods"

section that the pigs were weighed and blood samples collected each week but no results of the measurements were included, other than the statement that leucopenia, anaemia and thrombocytopenia occurred. At autopsy, the alimentary tract lesions were very similar to those seen in our experiment, being concentrated in the mouth, caecum and colon. Bacteriology results were not reported, though clumps of bacteria and *Balantidia* were seen histologically in the gut lesions and evidence of widespread bacterial embolism was found in many tissues. In those of our pigs which had these gut lesions, very few bacteria were seen in frozen sections and cultural examination showed that the lesions were not associated with any bacterial pathogens.

Those pigs which died with haemorrhagic diathesis showed autopsy results similar to pig 41.

Skin lesions were described which were similar to those seen in pig 48, but alopecia apparently did not occur and there was no mention of foot lesions such as those seen in 48 or 49.

Lymphoreticular tissues were described as atrophied and congested, and lymph nodes in many of their pigs showed extensive haemorrhage. In our high- and medium-dose pigs, there was a marked decrease in relative weights of spleens and thymuses compared with those of the control pigs. Grossly, haemorrhage was suspected in the lymph nodes of 47, 49 and particularly 41 because of the red appearance of the nodes, but all lymph nodes in all four

animals were much heavier than those of the controls, which was surprising in the case of pig 48, whose nodes showed an ill-defined cortex and no gross evidence of haemorrhage.

The toxic effects of adriamycin already discussed are not unexpected in tissues such as bone marrow, lymphoid tissue, alimentary epithelium, skin and coronary band of the hoof, where there is a high turnover of cells. Van Vleet et al., (1979) were interested in the fact that intestinal lesions were most common in the colon and caecum. They suggested three reasons for this, firstly that the turnover time of porcine large intestinal mucosal cells is more rapid than that of small intestinal cells, secondly that the bacterial and protozoal flora of the large intestine makes the mucosa more vulnerable to invasion, and thirdly that the small intestinal epithelium has a greater ability to regenerate in the face of continual drug-induced injury.

Van Vleet et al., (1979) had many problems with intravenous injections and the tissue necrosis which occurred after perivascular extravasations. Although it is possible that the metabolism of the tranquiliser in some way might have affected the actions of adriamycin, it was considered that the advantages of relative immobility and vasodilatation that the tranquiliser produced far outweighed any theoretical disadvantages. As a result, no extravasation occurred, despite repeated injections, and sclerosis of the ear veins was avoided by flushing out the veins with saline.

Other toxic effects were reported by Van Vleet et al., (1979), the most important of which was cardiotoxicity, which occurred only in pigs which were given the lower dose levels and those which had prolonged survival.

Several of their pigs showed acute fibrinous pericarditis or hydropericardium. Fibrous adhesions were present in the pericardial sacs in treated pigs 47 and 49. However extensive similar adhesions were seen in the little control pig 39, and this post-mortem finding was fairly common in stunted young pigs in the piggery at this time. Since the lesions were old, isolation of microorganisms was never attempted, but a mycoplasma infection occurring in early life was suspected. It was unlikely therefore that these findings in 47 and 49 were related to adriamycin dosage. Low dose pig 45 had an excess of fluid in the pericardial sac and an irregularity of cardiac rhythm was detected in this animal, though the ECG tracing did not reveal any abnormality.

Kehoe, Singer, Trapani, Billingham, Levandowski and Elson (1978) administered adriamycin to 10 normal dogs. Four were given a high dose ($80\text{mg}/\text{m}^2$) on 3 consecutive days and were then destroyed. Six were given a lower dose ($25\text{mg}/\text{m}^2/\text{week}$) for up to 20 weeks. Cardiac monitoring revealed an increase in sinus rate which lasted several hours after infusion, ectopic dysrhythmias consisting of premature atrial and ventricular contractions, ventricular tachycardia and atrial fibrillation, and atrio-

ventricular block, but no constant alterations in QRS contour or amplitude or QT interval. High-dose dogs also exhibited a pronounced fall in arterial blood pressure within 5 minutes of the start of administration. They concluded that if their findings were also applicable to man, they could provide an explanation of sudden deaths of human patients treated with adriamycin. There was no consistent correlation between onset of dysrhythmia and either drug dosage or duration of therapy in the dogs and the more severe changes could well be life-threatening, particularly in patients with pre-existing conduction delays or coronary disease.

Alterations in ECG such as those observed by Kehoe et al., (1978) were not present in the traces from pig 47 and the low-dose pigs, and there was no clinical or autopsy indication of congestive heart failure in any of the treated pigs. The cause of death in pig 49 may have been the necrotising colitis seen at autopsy but the lesions were not particularly severe. Heart action in this animal must have been restricted by the fibrous adhesion between the pericardium and the left ventricle. However much more severe adhesions were present in pig 47 and caused surprisingly little inconvenience to the animal, though they may have been responsible for the irregularity of rhythm showed by the first ECG trace. It is tempting to speculate that the cardiotoxic action of adriamycin may have contributed to the sudden death of pig 49.

The results of this experiment were considered when planning a therapy trial in lymphosarcomatous pigs. Although a dose level of 2mg/kg was clearly toxic, it was decided to attempt treatment with this dose in two animals so that maximum antitumour response could be assessed, and to use the lower dose level for the other two pigs.

ADRIAMYCIN THERAPY

MATERIALS AND METHODS

Two pairs of pigs were used, and these were two castrated males from one litter which were 7 months old at the start of the experiment and a male and a female from another litter, which were 11 weeks old. All four were cases of lymphosarcoma, which were diagnosed before 8 weeks of age, based on clinical and haematological findings.

One pair were given 2mg/kg adriamycin intravenously, and the second pair were given 1 mg/kg. Since the higher dose was likely to cause toxicity problems, both pigs received 4 mg/kg prednisolone intramuscularly for 5 days, 1 week before the start of the adriamycin schedule. This was considered necessary because both had thrombocyte counts below 100×10^9 /litre as a result of the lymphosarcoma, and the results of the toxicity experiment had indicated that adriamycin would further depress their thrombocyte counts.

Apart from this, the pre-treatment procedure was as described in the general methods section for therapy experiments. Staging laparotomies were not carried out at this time. As in the experiment using normal pigs, haematological parameters measured were WBC counts and differential counts, RBC counts, PCV, haemoglobin, thrombocyte counts and urea levels. Serum levels of total protein, albumin and SGOT were measured at intervals, and total and free bilirubin if the SGOT was elevated or if the serum looked yellow.

Weekly weighing and blood sampling commenced 5 weeks before the start of adriamycin dosing in the case of the pair receiving the higher dose, and 3 weeks before in the case of the pair receiving the lower dose. As in previous therapy experiments clinical examinations included weekly measurements of the inguinal and precrural lymph nodes, and abdominal measurement at the level of the umbilicus.

Sensitisation and skin tests with DNFB were carried out at 30 days and 10 days before the first adriamycin dose, in pigs 192 and 194 only.

The dosing procedure was identical to that already described in the experiment using normal pigs, and as before dosing was carried out every 3 weeks. The low-dose pigs received eight treatments, the higher-dose pigs only survived two and three treatments respectively.

Actual doses in mg were as in Table 33 with total doses for each pig.

The third dose given to 3785 was reduced by 75%, in accordance with the recommendations of the adriamycin dosage instruction leaflet (Pharmitalia) for cases with elevated serum bilirubin (see Results).

During the treatment period, the high-dose pigs were bled at 6, 9, 13 and 21 days after the first injection and at 4, 7, 11 and 18 days after dosing thereafter. Since little toxic effect was expected in the 1mg/kg group, from the results of the experiment using normal pigs, 192 and 194 were bled at 5, 12 and 19 days after dosing. Weighing

TABLE 33

DOSES OF ADRIAMYCIN IN MG

Dosage Group	Pig	Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	Total
1mg/kg	192	23.0	32.5	39.5	42.5	50.0	60.0	67.0	75.0	389.5
1mg/kg	194	27.0	38.0	47.0	54.0	60.0	80.0	95.0	104.0	505.0
2mg/kg	3785	80.0	83.0	22.0	Dead	-	-	-	-	185.0
2mg/kg	3786	64.5	68.0	Dead	-	-	-	-	-	132.5

and clinical examinations were continued. Lymph node measuring was continued until the pigs reached 60 to 70 kg body weight, after which they were too heavy to turn over on to their backs.

Skin tests were repeated on pigs 192 and 194 after three and eight adriamycin treatments.

Twelve days after the last dose, one pig from the 1mg/kg group, the female 192, was destroyed by pentobarbital overdose. Both high-dose pigs were already dead. Autopsies were carried out as soon as possible after death.

Three weeks after the last dose of adriamycin, 194 was tranquilised as before, and then given halothane by mask until surgical anaesthesia was achieved. An incision was made through the scrotal skin and tunica vaginalis and the right testis and epididymis were removed, using an emasculator to crush and cut the spermatic cord. The skin was sutured with nylon. The tissue was examined to investigate the possibility of using this animal for breeding. No further adriamycin treatment was administered. Weekly weighing, clinical observations and blood sampling were continued until 7 weeks after the last dose, when it was considered that the pig had relapsed. A further DNFB skin test was carried out, then it too was destroyed and autopsied.

RESULTS

Clinical Examinations

Both higher-dose pigs died fairly soon after starting therapy, one, (3786) 19 days after its second dose, and the other, (3785) 14 days after its third dose. Both were advanced cases, which were deteriorating in condition at the beginning of the experiment. They were in poor body condition, with the typical "pot-bellied" appearance. Five days of prednisolone therapy, given during the week preceding the adriamycin therapy, produced similar effects to those previously described in Chapter I. General fitness and appetite improved and there was a slight shrinkage of superficial inguinal and precrural nodes in both animals (see Clinical Records, Appendix II). There was a decrease in abdominal measurements, which was also noticeable looking at the animals, from 85.5 cm to 77.0 cm in pig 3786 and from 92.0 cm to 82.5 cm in pig 3785. A slight body weight loss occurred - 2.7 kg in 3786 and 2.0 kg in 3785. Both animals had slight diarrhoea 2 weeks before starting adriamycin therapy, but this cleared up without treatment.

Pig 3786 remained well throughout the treatment period with a good appetite and no further deterioration in body condition, until a few hours before its death, when it suddenly became very listless. It was noted to be constipated and be uninterested in food, but no other clinical signs were apparent. Its rectal temperature was 40°C, which was a little higher than the range of 37

to 39°C previously recorded in this animal during treatment but not as high as 40.3°C, which was recorded one pretreatment week when there were no other clinical signs associated with it.

As stated previously, there was a decrease in abdominal measurement when prednisolone was administered. There was no increase in the week following the first dose of adriamycin, but in the next 2 weeks, there was a gradual increase. After the second dose, the abdominal measurement decreased again, this time 6 cm, increasing once more during the 5th week. This decrease in the circumference of the abdomen may have been simply a result of a decrease in food consumption or other cause. However, if the superficial lymph node measurements, taken at the same time, are examined, it will be seen that the same pattern occurred especially during week 4, after the second dose, when a decrease in length occurred in all four nodes measured, of between 3 and 5 mm.

Pig 3785 remained bright with a good appetite for the first 4 weeks of therapy. However, during the 2nd week after the second dose of adriamycin, it was noticed to be rather listless and not eating quite so well. When examined, it was found to have an ocular discharge and its mucous membranes were slightly yellow. This was more noticeable in the sclera of the eyes. By the next week, the jaundice was much more obvious. The third dose of the drug was reduced by 75% as previously mentioned, but

even so, by the next week, the 7th, the whole skin-surface of the pig was bright yellow. It was losing condition rapidly, was very miserable, and was hardly eating, and so it was decided that it should be destroyed on humane grounds.

Its rectal temperature stayed within the normal range for these pigs throughout the experiment. Plates 9 and 10 show this pig before and at the end of treatment. It can be seen from the latter plate, that at the end, it lost its "pot-bellied" appearance and became very "tucked-up".

This happened fairly suddenly during the 7th week as can be seen from the abdominal measurements in Appendix II. There had been a gradual increase in the first 6 weeks, after the decrease noted while on prednisolone, but during the 7th week this measurement decreased by 20 cm from 89 cm to 69 cm.

When the lymph nodes of 3785 were examined, it was clear that there was a definite shrinkage of these nodes after each dose of adriamycin. Comparing the measurements with those obtained in week 0, this was slight after the first dose because the nodes had already been reduced by the prednisolone treatment. However the effects of the prednisolone would be expected to have disappeared by the time the second dose of adriamycin was administered. If the measurements taken the 1st week after the second dose are compared with those taken before prednisolone in week 1, by multiplying the length and breadth of each node as previously described in Chapter I the reduction was from

1710 to 1200 (510), from 1848 to 1104 (744), from 760 to 448 (312) and from 798 to 504 (294), giving an overall mean decrease for the inguinal and precrural nodes of 37%. After the third dose, only the inguinals could be measured with any accuracy, but using the same method, the mean decrease in size was 43.6% compared with the measurements in week-1.

Weight Gains - Higher Dose Pigs

Pig 3786 showed no improvement in weight gain with therapy. Its body weight increased and decreased so that there was only 4.5 kg actual gain in weight during the whole period of the experiment. Its weight at death was 0.5 kg lighter than at the start of treatment.

During the first 5 weeks of therapy, pig 3785 gained 5 kg in weight. However, after that, it lost weight rapidly and when weighed before post-mortem it was found to have lost 15 kg in 2 weeks. This was obvious from its appearance see plates 9, 10.

During the pre-treatment period, both low-dose pigs suffered from an upper respiratory infection, which developed into pneumonia. The female made a satisfactory recovery after treatment with ampicillin for 5 days. However, the male continued to have difficulty in breathing due to a chronic rhinitis, particularly when tranquilised, and auscultation of the thorax revealed abnormal lung sounds, particularly high-pitched rhonchi, for a considerable time. Periodic treatment with antibacterial drugs was

necessary, and atropine sulphate was administered with each dose of tranquiliser. On one occasion, oxygen was given by mask to relieve severe respiratory distress.

Both low-dose pigs survived eight doses of adriamycin, and during this time their body condition improved greatly. By the end of the treatment, both animals looked much like normal pigs, though slightly small for their age, and the male, 194, was easily fat enough to be mistaken for a bacon pig (see plates 11 to 14).

Apart from the recurrent upper respiratory problem previously described, male 194 remained well and there was little to note clinically. It developed small ulcers on the gum round the base of the lower incisor teeth during the 11th week of treatment, but these healed rapidly and did not recur. Vomiting occurred only once, 5 weeks after the last dose of adriamycin. The chronic rhinitis caused severe dyspnoea particularly during the 13th week of treatment, when a febrile response was recorded and the pig lost its appetite. This responded to intramuscular chloramphenicol. Since it was considered likely that the adriamycin treatment was reducing the pig's resistance to infection, a trimethoprim/sulphonamide preparation ("Tribrissen", Burroughs Wellcome) was given in food for 6 days after the following two doses of adriamycin, the sixth and seventh. Although its respiration was still noisy, it remained fit and lively. "Tribrissen" was not given after the eighth dose, and once again the pig became rather listless and developed a slight cough.

At the end of treatment, one testis was removed under halothane anaesthesia to test for sexual maturity. The epididymis was almost replaced by hard, white cellular tissue and no normal spermatozoa were identified in the small amount of fluid from the epididymis.

By 4 weeks after the last dose of adriamycin, the pig was starting to lose condition, and by 6 weeks, its skin was pale and its abdomen was becoming more pendulous. Its rectal temperature rose to 40.7°C and it showed little interest in food. At this stage, the decision was made to destroy it. The abdominal measurement of pig 194 decreased slightly from 75 cm to 71 cm the 1st week after the first dose of adriamycin and from 84 cm to 82.5 cm after the third dose. Apart from these occasions, its abdominal measurements increased steadily, until after the fifth dose it became too heavy to turn over on to its back. It was decided that measurements made any other way would be unreliable and so they were stopped. However plates 13 and 14 of 194 before and during therapy illustrate that the enlargement of the abdomen, typical of the lymphoma pig, was no longer obvious, and increase in abdominal measurements would certainly have been due to growth and deposition of fat.

It was only possible to measure the inguinal lymph nodes of 194 with reasonable accuracy and these measurements were also stopped after the fifth dose of adriamycin. There was a slight shrinkage of the nodes the 1st week after each dose, as had occurred with the two higher dose

pigs. The nodes increased in size again during the 2nd and 3rd weeks after each dose. However, the nodes must have been decreasing in size relative to the size of the animal as the measurements noted after the fifth dose were very similar to those taken during the last pretreatment week (56mm x 32mm compared with 56mm x 29mm, and 57mm x 34mm compared with 53mm x 31mm) even though the pig had increased in body weight from 26.5 kg to 63.5 kg in that time.

The female 192, which was also given 1 mg/kg adriamycin for eight doses, developed a high fever (41.9°C) during the 8th week of treatment. It was anorexic and vomited once. As several animals in the piggery were showing similar signs, it was suspected that erysipelas might have been introduced and all were given intramuscular injections of a solution of crystalline penicillin immediately and procaine penicillin for 3 days. No further signs of erysipelas developed but all the pigs which had been ill, including 192, improved rapidly and three days later were fit and eating normally, and rectal temperatures were within the usual range. After this, 192 remained well until after the fifth dose of adriamycin. During dosing, the drug was accidentally injected into the auricular artery, lying beside the central branch of the posterior auricular vein. The error was soon noticed as the whole pinna became red and half-an-hour later a mottled purple. The irritant nature of the drug became apparent over the next 3 weeks. After 2 to 3

days, the ear was very swollen though seemed to be more pruritic than painful. The edges of the pinna started to curl over after 7 days and the skin was blistered. After 21 days the skin lesions had dried up and healed, and the scabs had mostly fallen off, leaving the ear curled and distorted. Pruritis was still evident (see Plate 15 to 17).

During the 14th week of treatment, and again during the 17th week, this pig suddenly collapsed and for about an hour was unable to rise. Rectal temperature was normal and the heart, though the rate was rapid, sounded normal on auscultation. At these times, it was not interested in food or in its surroundings. As there appeared to be no clinical explanation for the collapse, it was assumed that the animal had slipped in its pen and fallen heavily. On both occasions, it recovered without treatment, though it was unsteady in gait for several hours.

192 was destroyed 10 days after the eighth dose of adriamycin, so that the maximum effect of the drug could be shown at autopsy.

Abdominal measurements decreased slightly after the first dose from 68 cm to 65 cm, after the second dose from 75 cm to 73.5 cm and after the third dose from 80.5 cm to 79 cm. After this, they increased steadily as its body condition improved, until they were stopped after the 15th week of treatment when it became too heavy to turn over. Inguinal lymph node measurements shrank

slightly after each dose of adriamycin as in the other treated pigs and became difficult to carry out because of subcutaneous fat. In the 2nd week after the fifth dose, the nodes were impossible to measure. The week before this, as with the other low-dose pig, the measurements were almost identical to those obtained just before treatment started (46 mm x 20 mm compared with 45 mm x 20 mm and 41 mm x 20 mm compared with 44 mm x 20 mm) even though the pig's body weight had increased from 21.5 kg to 53.5 kg.

Electrocardiograph tracings from both low-dose pigs and the end of treatment showed a perfectly regular pattern. The intervals between QRS complexes were measured in millimetres on all parts of the traces from leads I, II and III from pig 192 and lead I from pig 194. The intervals in pig 192 were 18.5 to 19 mm, and in pig 194 18 to 18.5 mm, without exception.

However when the height of the QRS complexes were measured (leads II and III) from pig 192 they were found to vary from 10 mm to 17 mm. The only satisfactory trace (lead I) from pig 194 did not show pronounced QRS complexes but if they were measured they varied between 6 and 8 mm. This variation might have been magnified on the other lead tracings, but the pig's rapid jerky respiration made them impossible to follow. To ascertain whether this variation in height of QRS complexes was caused by the drug it would have been necessary to examine a pretreatment trace. No other abnormality was

seen on traces from either low-dose pig.

Weight Gains Low-dose-pigs

During the treatment period, and for 3 weeks after the last dose of adriamycin, pig 194's weight gain was excellent for a lymphosarcomatous ^{pig}/reaching 90 kg (bacon weight) by 214 days of age. Since lymphosarcoma was first diagnosed in the piggery, 28 untreated cases survived 30 weeks or over. Of these 28 cases, records of weight gain were kept for 17. Only 3 cases reached 90 kg by 30 weeks of age. The weight gain of 194 was, however, still not as rapid as that of a normal animal. Figure 16 illustrates the body weight gain of this animal and of the other low-dose pig, 192, compared with maximum and minimum weight gains of a normal, closely related litter.

Although 192 did not gain weight quite as rapidly as 194, its weight at 30 weeks was 60 kg. Records of untreated cases show that 8 out of 17 reached 60 kg at 30 weeks.

TABLE 34

ADRIAMYCIN THERAPY

DNFB Skin-tests-results:

Pig No.	At sensitisation	Pre-treatment	During treatment		Post-treatment
			1	2	
192	0	1	2	2	Dead
194	0	0	2	2	1

1 - after three treatments

2 - after eight treatments

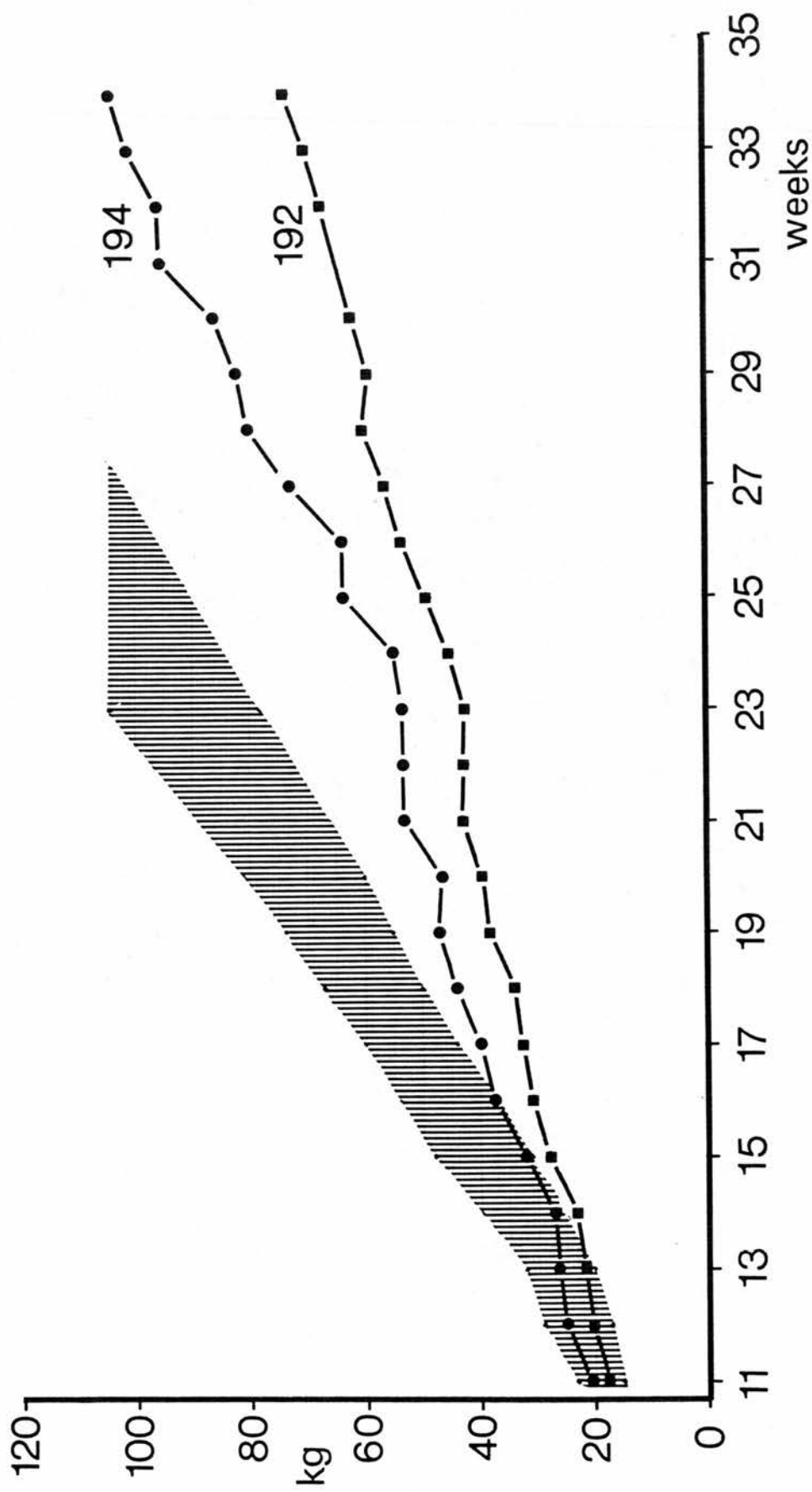


FIGURE 16

Body weight gains of two lymphosarcoma cases treated with 1mg/kg adriamycin compared with range of weight gains of a normal closely-related litter.

DNFB Skin-tests

From table 34 it can be seen that before treatment started, 194 was anergic to DNFB and 192 showed only a very slight response. During treatment, the reaction scores increased to 2 in both animals. It would appear that, as in the first prednisolone therapy experiment, treatment with adriamycin temporarily improved the cell-mediated response, though it was still poorer than would be expected in normal, untreated pigs.

Haematology Results

Higher-dose pigs (2mg/kg)

Total WBC counts were low in both pigs at 6 and 9 days after the first dose of adriamycin, increased between 13 and 21 days and decreased again after each subsequent dose (See Appendix Tables 36 and 37). When absolute mononuclear cell and neutrophil counts are considered independently, the results are shown in figure 17. Absolute mononuclear cell counts fell with prednisolone and still further with each adriamycin dose. The nadir was at 6 days after the first dose. After the second and third doses these pigs were bled at 4, 7, 11 and 18 days and the nadir was at 4 days, with a terminal fall at 11 days in 3785. Absolute neutrophil counts did not show a consistent pattern in these pigs. After the first dose, there was a fall in the counts at 9 days, though in 3786 a similar fall had occurred spontaneously before the start of treatment. After the second dose, the counts dropped slightly at 4 days, then increased

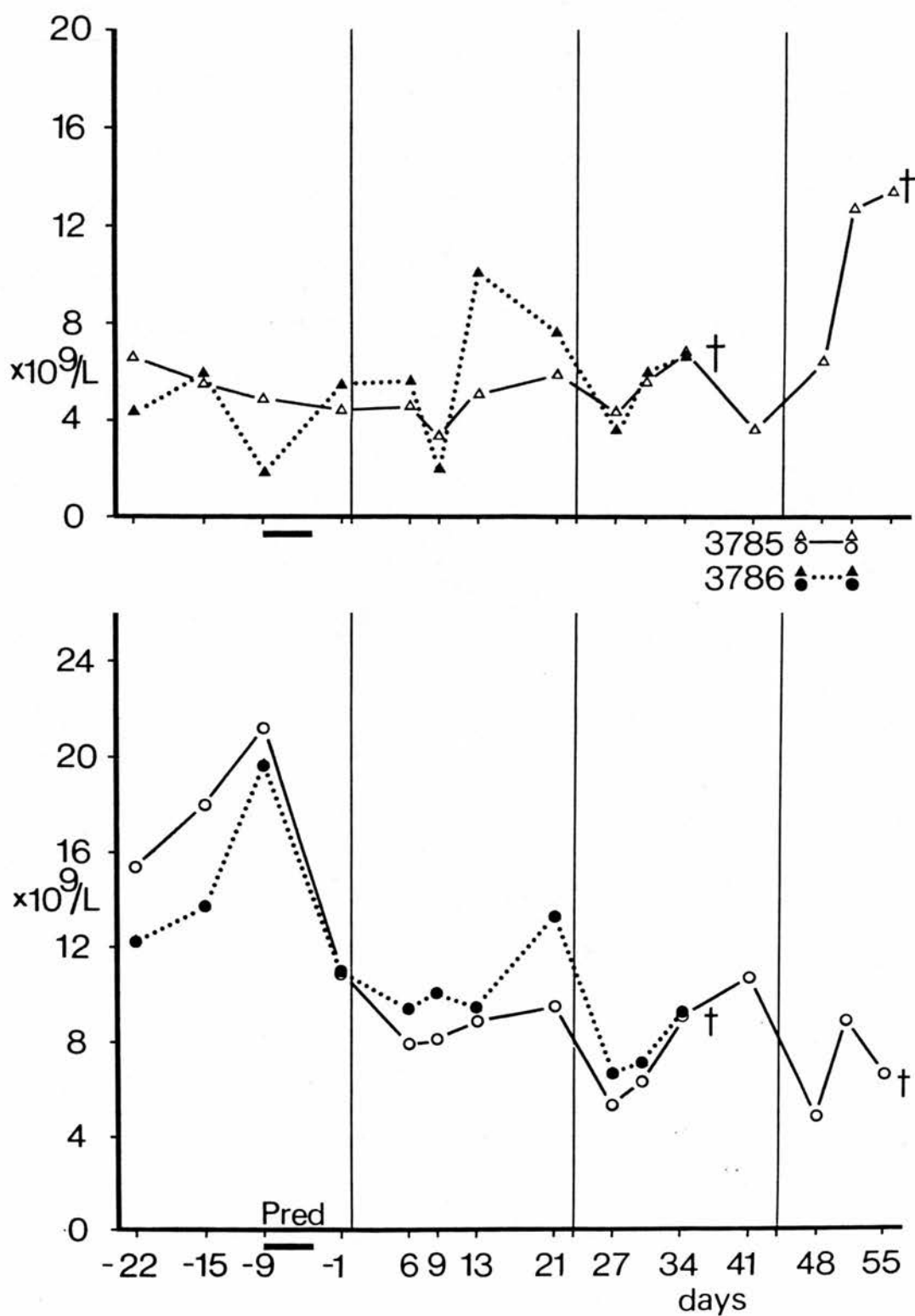


FIGURE 17

Absolute neutrophil counts (above) and mononuclear cell counts (below) of two lymphosarcoma cases treated with 2mg/kg adriamycin. Vertical lines indicate times of dosing.

239

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and fell again at 18 days. After the third dose, the absolute neutrophil count in 3785 rose sharply as the pig's condition deteriorated.

RBC counts, PCV and Hb concentration (Appendix I I) followed almost parallel courses in both higher-dose animals, falling progressively in weeks - 4 to - 1, rising with prednisolone treatment, and then after dosing with adriamycin, maintaining a fairly constant level until the 5th week of treatment. Pig 3786 died 6 days after it was blood-sampled on day 34. After this, RBC counts, PCV and Hb concentration of the remaining pig 3785 rose sharply, reaching values of 7.06×10^{12} /litre, 0.40 and 13.7 g/dL respectively on day 51 and falling again slightly before it was destroyed on day 56. From these figures, it might be assumed that the drug had caused the red cell picture to return to normal, but as the animal was severely ill at this point, dehydration may have confused the results.

When the derived red cell indices are considered, it appears that there was no effect on MCH which could be associated with adriamycin dosage, but MCV decreased after each dose. There was little overall change in MCHC from beginning to end of treatment in either animal, but there was a decrease in MCV from 58.4 fl before treatment to 51.7 fl in 3785, and from 65.7 fl to 55.0 fl in 3786

Thrombocyte counts in both pigs increased rapidly

with prednisolone therapy but were not affected by 2 mg/kg adriamycin. Counts ranged from 86 to 237×10^9 /litre in 3785 and from 160 to 239×10^9 /litre in 3786, but the fluctuations could not be attributed to drug dosage.

Lower-dose pigs (1mg/kg)

Total WBC counts were lowest in both pigs at 7 days after the first dose of adriamycin and at 5 days after each subsequent dose (See Appendix II) Figure 18 clearly shows the effect of dosing on absolute mononuclear cell counts, particularly in pig 194. From day 40 onwards, this animal appeared to be going through a leukaemic phase of the disease, which was certainly controlled, though not prevented, by three weekly dosing with adriamycin. Only six doses are illustrated but the same pattern was repeated after doses seven and eight in both animals (See Appendix II).

Figure 18 also shows absolute neutrophil counts of both pigs. As the results from the higher-dose pigs showed, there was no consistent pattern which could be attributed to dosing with adriamycin, but the counts remained low throughout the treatment period, especially towards the end of treatment. The lowest neutrophil count was 0.8×10^9 /L, which was recorded 5 days after the sixth dose in 194 and 6 days after the eighth dose in 192. This depression of neutrophil counts was almost certainly due to adriamycin.

After the last dose of adriamycin in 194, there was

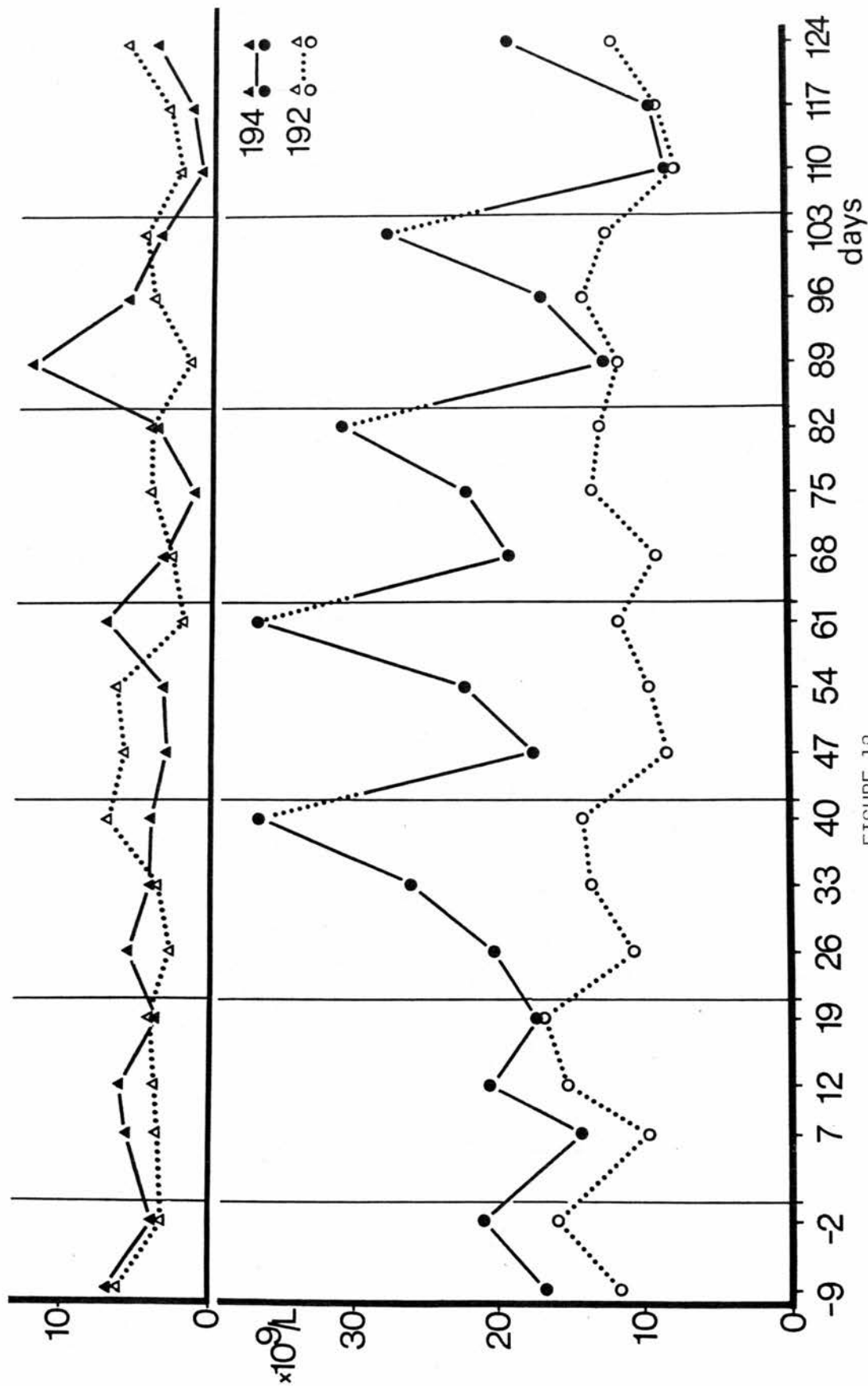


FIGURE 18

Absolute neutrophil counts (above) and mononuclear cell counts (below) of two lymphosarcoma cases treated with 1mg/kg adriamycin. Vertical lines indicate times of dosing.

a rapid increase in total white cell counts and this was due to an increase in mononuclear cells.

The low-dose pigs were only mildly anaemic before the start of dosing. RBC counts, PCV and Hb values during treatment revealed no repeated effect which could be interpreted as a result of adriamycin treatment. Neither was there any overall worsening or improvement in the red cell picture during the whole treatment period in either animal (See Appendix II). Though there were fluctuations from week to week, these were more likely to be manifestations of the state of activity of the disease. They were more noticeable in 194 and the lower RBC counts, PCV and Hb values were recorded between days 47 and 110, a period which corresponds with the "leukaemic" phase indicated by the white cell picture in this animal.

The same conclusions can be drawn from the calculated values of MCV, MCHC and MCH.

After treatment was stopped, the pig which was left alive showed a decrease in RBC counts, PCV and Hb, from four weeks after the last dose until it was destroyed. This corresponded with the clinical evidence of relapse and rapid tumour growth during this period.

Thrombocyte counts were also unaffected by 1 mg/kg adriamycin. Counts were within the range of values for normal pigs before the start of treatment, for 15 weeks during treatment in pig 192 and for 4 weeks of treatment in 194. Thereafter, low counts ($< 100 \times 10^9/l$) were frequently obtained in both animals, but these low

counts were almost always associated with severe clumping of thrombocytes. Often this was due to delay in obtaining the blood samples as the pigs grew larger and more difficult to handle, but sometimes it occurred even when there were no sampling problems. It is unlikely, therefore, that these low counts were an indication of thrombocytopenia (See Appendix II).

S.G.O.T. - Higher dose pigs 3785 and 3786 only

See Table 44, Appendix II.

Pretreatment levels of SGOT were higher than normal particularly on days -15 and -9. However there was a fall in both animals after prednisolone therapy. During adriamycin therapy, SGOT levels stayed within the normal range in 3786 until it died, but in 3785 by day 34, it had risen to 119 iu/litre. At this time, though the pig still looked normal, the serum was slightly yellow and it was thought advisable to measure serum bilirubin. It was also measured in 3786 for comparison and there was little difference, both levels of bilirubin being on the low side of the normal range. However, by day 41, the SGOT level in 3785 was 210 iu/L, the pig was clinically jaundiced and total serum bilirubin had risen to 73.9 $\mu\text{mol/l}$. Free and conjugated bilirubin were almost equal (35.6 and 38.3 $\mu\text{mol/l}$ respectively). On day 48, SGOT had fallen slightly to 149.3 iu/l, but total serum bilirubin had risen to 208 $\mu\text{mol/l}$. Free bilirubin had increased only slightly but conjugated bilirubin accounted

for 168.1 $\mu\text{mol/l}$. By day 55, SGOT had risen again to 181 iu/l , and the serum was brown with a total bilirubin level of 279 $\mu\text{mol/l}$. The free bilirubin had risen to 110 $\mu\text{mol/l}$ and conjugated bilirubin was unchanged at 169 $\mu\text{mol/l}$.

On days 34 and 41 when SGOT was first found to be increased in pig 3785 serum glutamic pyruvic transaminase (SGPT) was also measured, though it was too expensive to carry out measurements of both of these enzymes routinely. On day 34, when SGOT was 119.4 iu/l , SGPT was 83.4 iu/l . On day 41, when SGOT was at the highest level measured, 210 iu/l , SGPT was only 42.2 iu/l , though this was still higher than the normal range.

Blood Urea (See Tables 40 to 43 Appendix II).

Blood urea levels were within the normal range throughout the experiment, except in pig 3785 which had slightly elevated levels before treatment started, and at the end of treatment, particularly on day 48, when it was jaundiced.

Serum proteins - higher-dose pigs 3785 and 3786

See Table 45, Appendix II.

Changes in serum proteins in pig 3786 during treatment were slight. There were small increases in total protein and albumin and a very small decrease in globulins.

In pig 3785, the most dramatic change was a progressive fall in serum albumin and in the albumin/globulin ratio from day 34 to the end of treatment. This corresponded with the clinical loss of condition, appearance of

jaundice, and raised SGOT and bilirubin levels. Globulins fell at the start of treatment but rose markedly at the end of treatment.

Serum Proteins - Low-dose pigs 192 and 194

During the course of treatment, in both 192 and 194, serum albumin rose gradually from a fairly low level of around 25g/L, to around 40 g/L in the week after the last dose of adriamycin (See Appendix II). In the surviving pig 194, it reached 49.4 g/L the following week, and then fell again to 32.2 g/L as the animal's condition deteriorated.

Total serum globulin was calculated as before by subtraction of albumin from total protein measurements. There were wide fluctuations in total globulin levels during the treatment period, but these did not appear to be related to adriamycin dosing. Pig 194 showed a marked rise in serum globulin as it relapsed after the end of treatment (See Appendix II, Table 46.)

The albumin/globulin ratio increased at the end of the treatment period, especially in pig 194 in which it was greater than unity from the 2nd week after the sixth dose onwards. After the eighth dose in this animal, it rose to a peak of 2.23, and then fell to 0.69 as it relapsed (See Appendix II.)

Post-Mortem Results

High-dose pigs - 2 mg/kg adriamycin

3786 castrated male

This was a well-developed, well nourished carcase.

The thymus was found in the neck as a narrow ribbon of whitish-yellow small firm nodules. The thoracic thymus was less distinct.

There was about 50 ml of clear fluid in the pericardial sac. The heart was small and the muscle flabby. The right ventricle was 8 mm thick, the left ventricle was 18 mm thick. The muscle and valves were normal.

The liver was uniformly slightly pale, pinkish-brown in colour. On close inspection this was due to a pale outlining of the lobules. There were no focal lesions.

The edge of the spleen was bent over on to the parietal surface along one border and there were fibrous adhesions between the two surfaces of the spleen. Both the hilar and non-hilar surfaces showed numerous 5-15 mm sessile nodules. On cut surface, the spleen showed red faintly lobulated tissue with indistinct 2 mm areas of white pulp. The nodules were dark red/white mottled and had a sharp border but no capsule. Some of the larger nodules showed yellow dry central necrosis.

The surface of the duodenum was red and corrugated, the mucosa being slightly thickened.

The small intestine was mostly empty. The wall was flushed in some regions. In the ileum, the mucosa was covered by an incomplete yellowish-brown fibrin cast. It was corrugated and thickened up to 3 mm by white tissue in the mucosa and submucosa, and in some places there were, on cut surface, yellow ulcers penetrating down to the

muscle coat. The mesentery of the small intestine was oedematous.

The caecum was full of rather fluid finely fibrous material. The mucosa was greyish-brown in colour and probably necrotic, but the wall was not markedly thickened. This reaction continued for the first coil of the colon, but then the contents became more solid, the mucosa was normal and pale in colour with occasional brown plaque-like nodules, probably lymphoid masses. There was marked submucosal oedema in the caecum, becoming less in the colon, and there was marked oedema in the serosal connective tissue between the coils of the colon.

The lymph nodes showed a mottled pink and white or fawn and white pattern, with no distinct cortex and medulla on cut surface, and there was haemorrhage in some of them.

3785 - Castrated male

This was a very thin pig with sunken flanks, not pot-bellied, and the bony prominences were very distinct.

There was marked yellow jaundice of the skin. There were no subcutaneous petechiae when skinned.

There was only a small red translucent sheet in the neck and anterior mediastinum at the site of the thymus.

The lungs were not quite fully expanded but there were no focal lesions. The heart was rather small. The right ventricle was very thin-walled (2 mm) dilated with non-clotted blood. The left ventricle was rather thin (10 mm). The valves and muscle colour were normal. The major arteries were very jaundiced and the fat in the

coronary grooves was translucent, yellow and jelly-like.

The liver was very large. The borders were slightly rounded, the lobulation distinct but the thickness of interlobular septae not excessive. The cut surface showed congested lobules with prominent interlobular septae. The consistency of the surface and cut surface was increased. The gall-bladder contained only about 10 ml of clear pale yellow fluid but this ran out into the small intestine lumen with gentle pressure, indicating that the cause of jaundice was intrahepatic.

The dorsal end of the spleen showed a folding of one edge on to the parietal surface where it was fixed by fibrous tissue. The cut surface was deep red, faintly nodular but with no clear cut white pulp. At the dorsal end, there was a raised yellow 2-3 cm nodular mass, which was roughly wedged-shaped on cut surface and composed of dry yellow necrosis.

The stomach contained only a small amount of bile-stained meal and fluid. The oesophageal region and cardia were normal. The wall at the pylorus was thickened, on cut surface half muscle and half white, soft, cellular submucosa.

The small intestine was normal. The large intestine showed marked dilatation with the colon full of normal faeces becoming very hard and dry covered with a coat of mucus, suggesting some degree of gut stasis.

The fat round the viscera was atrophic, like translucent jelly.

All the lymph nodes on cut surface showed a green soft cellular tissue exuding fluid separated into lobules by thin white trabeculae, no distinct cortex being visible, except the mesenteric and colonic nodes which showed small groups of white, soft, cellular, cortical nodules centrally. The gastric lymph node showed red haemorrhagic tissue in the centre.

The bone marrow of the femur was dark red and blood-like, and translucent i.e. congested serous atrophy.

Low-dose pigs - 1 mg/kg adriamycin

192 - female

This was a well-developed, well-nourished carcase.

Both ears showed scabs resulting from perivascular injection of the drug.

The border of the right ear was curled over.

The nasal mucosa was congested but there was no rhinitis. No ulceration was observed in the mouth. The tonsils were slightly thickened and on cut surface showed white lobulated tissue.

The thymus was of moderate size, both in the neck and in the chest.

There was a moderate amount of serous fluid in all three cavities but this may have been due to difficulty in destroying the animal.

The diaphragmatic surface of the liver showed one linear scratch 2 mm wide and 4 cm long not extending into

the underlying tissue. The capsule was intact. There were some ill-defined pale lobules scattered throughout the surface and cut surface.

The kidneys showed a few 1 - 2 mm white nodules, visible on the surface and cut surface of the cortex. The capsules stripped easily.

The stomach showed an ill-defined shallow, dry, yellow ulcer in a semi lunar shape, 3 cm long by 1 cm wide, at the junction between the oesophageal region and the fundus.

The duodenal mucosa was dull red with prominent folds. The cut surface showed that the mucosa was thickened up to 5 mm by creamy white firm cellular tissue.

The remainder of the small intestine was normal. Peyer's patches were prominent due to a thickening of the mucosa by white cellular tissue, often forming ridge-like patterns.

Lymphoid nodules in the large intestine were prominent. All lymph nodes showed a white/fawn, almost flat field of firm tumour tissue with some lobulation but no medulla, except in the iliac and prescapular lymph nodes which had a 1 mm brown medulla. The gastric node showed petechiation.

The spleen was grossly enlarged and its surface was distorted by some degree of folding and by 2 cm sessile nodules along the hilus. On cut surface the spleen was red and cellular, exuding blood, showing lobulation but no distinct white pulp. The nodular lesions were cellular

with no white pulp. They had a distinct edge but no capsule.

The ovaries showed multiple 2-4 mm clear cysts.

The bone marrow of the femur was reddish-pink and floated heavily in formalin.

Low-dose pigs - 1 mg/kg adriamycin

194 - entire male

This was a well-developed but rather thin carcase.

The nose was bilaterally symmetrical, but with Phloxine-Alcian Blue staining, however, there was a patchy red colouration of the majority of the turbinate mucosal surfaces indicating an abnormality in the mucosa (Done, 1976).

The ears showed two 3 cm diameter scabs, one of which on cut surface extended 5 mm down to the cartilage, the result of the drug being injected outside the vein.

The thymus was composed of small white/fawn lobules of cellular tissue.

Both ventricular walls of the heart were of equal thickness, 2.5 cm, and were rather flabby. The lungs showed no abnormality.

The liver was large and slightly friable, with distinct lobulation. The periphery was pale.

The pole of one kidney showed a series of small white scars depressing the surface, and extending towards the medulla on cut surface. Otherwise the kidneys showed no abnormality.

The spleen was large. The hilus showed some 1 - 2 cm sessile nodules. The cut surface was red and cellular with ill-defined white pulp. The nodules were white and cellular with discrete borders but no capsule.

The stomach showed a broad crescent 3.5 cm x 6 cm of green dry ulcerated slightly depressed lesion in the oesophageal region. The wall under this was slightly thickened by fibrous tissue. In the fundus there were a few 1 - 2 mm x 5 mm long red ulcers.

The small intestine was normal but the mucous membrane of the duodenum and the Peyer's patches of the ileum were thicker than the rest. The large intestine showed slight lymphoid follicular hyperplasia.

The bone marrow of the femur was red and cellular throughout the whole of its length, with white cellular foci - this tissue sank in formalin.

Only one testis was present, the other having been removed. The testis was brown in colour on cut surface. The head and body of the epididymis were larger than normal and replaced by hard white cellular tissue.

All lymph nodes showed a flat field of fawn cellular tissue, except the prescapular, iliacs and popliteal which showed a 1 mm brown medulla and the splenic which showed up to 4 mm of red medulla. The mesenterics showed occasional cysts of 2 - 5 mm containing clear fluid.

Relative weights of lymph nodes and organs

Table 35 shows the relative weights of lymph nodes and organs of the treated animals compared with those of three untreated normal pigs and 10 untreated lymphosarcoma cases of similar ages.

In pig 3786, which died after two higher-dose adriamycin treatments, the results show that the drug did not reduce the relative weights of any node or organ below the minimum value of the 10 untreated cases, with the exception of the spleen.

In pig 3785, which had three treatments, the mesenteric and colonic nodes were reduced to less than half of the mean value for the 10 untreated cases. The gastric and splenic nodes were reduced to well below the minimum value of the untreated cases but were still about 50 times larger than those of the normal pigs. The bronchial nodes and spleen were also much reduced in weight. The superficial nodes were not much affected. The liver was larger than in any of the untreated cases and was more than four times larger than the heaviest normal liver. The adrenals were also large.

The relative weights of lymph nodes of 192 and 194 were similar to those of untreated cases, and, surprisingly those of 192 which was killed at the end of treatment, were larger than those of 194, which was allowed to relapse. The thymus of 192 was more than twice as

large as the mean value for untreated cases, and also larger than in the normal pigs.

TABLE 35

ADRIAMYCIN THERAPY		NORMAL Range of Three Untreated Pigs	LYMPHOSARCOMA CASES		
Relative Weights in cg/kg			TREATED		UNTREATED
			Low Dose (1mg/kg)	High Dose (2mg/kg)	Mean of 10 and Range
Pig Nos			192	3785	3786
Age at Death (days)	250 - 309		255	302	286
Body Weight (kg)					288.5 (249 - 313)
Pre-Treatment			21.5	40.0 ⁺	32.3
End of Treatment	78 - 179		77.0	28.5 ⁺	34.5 [*]
End of Experiment			-	-	-
Lymph Nodes					
Mesenteric and Colonic	96 - 103		2208	1509	4307
Gastric and Splenic	12 - 14		1592	615	1262
Bronchial Group	3 - 4		89	38	98
Total Splanchnic	111 - 121		3889	2162	5667
Head Group	11 - 16		149	94	184
Iliac Group	2 - 12		26	38	33
Prescapular	1 - 5		2	13	17
Precrural	4 - 6		5	13	27
Inguinal	3 - 9		13	38	48
Popliteal	1 - 1		3	5	20
Total Superficial	22 - 49		198	201	329
Thymus	8 - 40		65	14	34
Spleen	127 - 192		2097	723	835
Liver	614 - 2205		3831	9221	5583
Kidney (mean)	128 - 186		208	397	383
Adrenal (mean)	2 - 15		7	19	14

⁺ Destroyed
After 3 treatments

^{*} Died after
2 treatments

DISCUSSION

The higher dose level (2mg/kg) of adriamycin appeared to be fairly effective in suppressing the tumour, particularly in pig 3785. Shrinkage of lymph nodes, as shown by the measurements of the superficial nodes and the relative weights of nodes at autopsy, suggested that a good remission might have been attained if it had been possible to continue the experiment. However this dose level was even more rapidly toxic in the lymphosarcomatous animals than it had been in the normal pigs, and the sudden death of 3786 with necrotising enteritis was probably a direct result of the therapy. The terminal jaundice of 3785 raised the question of whether adriamycin could be hepatotoxic in the pig. The predominance of conjugated bilirubin on day 48 suggested an obstructive cause of the jaundice. However at autopsy there was no obstruction of the bile duct, therefore the obstruction must have been within the liver. The rise in SGOT and SGPT levels, though not dramatic, was likely to be due to damage to liver cells. The gross appearance of the liver indicated that the whole organ was involved.

Benjamin (1975) listed 11 side-effects of adriamycin therapy in human patients, but hepatotoxicity was not among them. Hepatopathy was reported after adriamycin dosage in two patients by Kun and Camitta (1978), but this occurred only after irradiation. The enhancing effects of adriamycin on tissue damage after irradiation has been documented by other authors. There was no

suggestion by Kun and Camitta (1978) that the hepatopathy was a direct effect of adriamycin.

It is recognised that liver dysfunction may increase markedly the other toxic effects of adriamycin, because it delays the metabolism and elimination of the drug, resulting in prolonged and higher blood levels. Elevation of serum bilirubin is the indication to decrease the dose of adriamycin administered by 50% or even 75% (Benjamin, 1975).

Pretreatment blood urea and SGOT levels were slightly higher than the normal range in 3785, although serum protein levels were normal, therefore it is possible that liver function was already impaired e.g. due to severe tumour infiltration, or previous toxic damage, though the latter possibility was extremely unlikely. If this were so, it is surprising that there were not more marked adriamycin - induced effects on the red cell picture, thrombocyte counts and gastro-intestinal tract after the first two doses.

The results obtained from 192 and 194, which were given 1 mg/kg adriamycin, suggest that this dose level suppressed the tumour temporarily after each administration but that the tumour recovered rapidly. However, despite the fairly poor response both pigs remained very fit, and their growth rates were spectacular for lymphosarcoma cases. As the normal pigs had shown previously, this dose level was not associated with any

clinical signs of toxicity, until the unfortunate intra-arterial injection of the fifth dose. The disastrous effects on the ear were similar to those occurring after perivenous extravasation. The effects of this kind of accident in human patients were described by Reilly, Neifeld and Rosenberg (1977). The tissue damage caused by adriamycin may progress from painful oedema and erythema to full-thickness skin necrosis, often with involvement of underlying tissues such as tendons and nerves, resulting in ulcers, which heal slowly or not at all, and even joint contraction with loss of function. They recommended immediate injection of hydrocortisone and the application of ice packs to the site, but it was felt that these measures would cause the pig further distress as it very much resented handling of the ear. Similar damage to pigs' ears was described by Van Vleet et al., (1979), caused by adriamycin extravasation.

Both low-dose pigs had large ulcers in the oesophageal region of the stomach and 194 also had ulcers in the fundus. These findings were not uncommon in older lymphosarcoma cases and may not have been associated with the treatment.

Adriamycin appeared to suppress the thymus in the normal pigs. However, the large size of the thymus in 192 suggested that this effect was counteracted by the effect on the tumour and the improvement in body condition.

The antitumour effect of the drug may not have been dramatic, but it still suppressed the tumour at the end of the treatment period of 150 days, therefore there was no indication of resistance developing. Carter (1975) quoted a short response duration in human lymphoma cases, to whom adriamycin had been administered as a single agent, but these were patients who had relapsed on conventional therapy. It would have been interesting to use adriamycin in combination with other drugs in the pigs, as it is now an accepted component of therapy protocols for lymphoma in humans (See Introduction to this Chapter). As a single agent, its toxicity made it impossible to administer a really effective dose.

CHAPTER III

CYCLOPHOSPHAMIDE

INTRODUCTION

Cyclophosphamide is the most commonly used example of the group of antitumour drugs known as the alkylating agents. Hill (1975) wrote a detailed review monograph about cyclophosphamide. In the introduction, he stated that probably more individual doses of cyclophosphamide are given to cancer patients than any other drug, due to the fact that this agent is effective against more different types of human neoplastic disease than any other chemotherapeutic compound. Since its discovery, until 1975, it had been mentioned in 10,000 different publications, and at least 28 different names have been applied to it in different parts of the world.

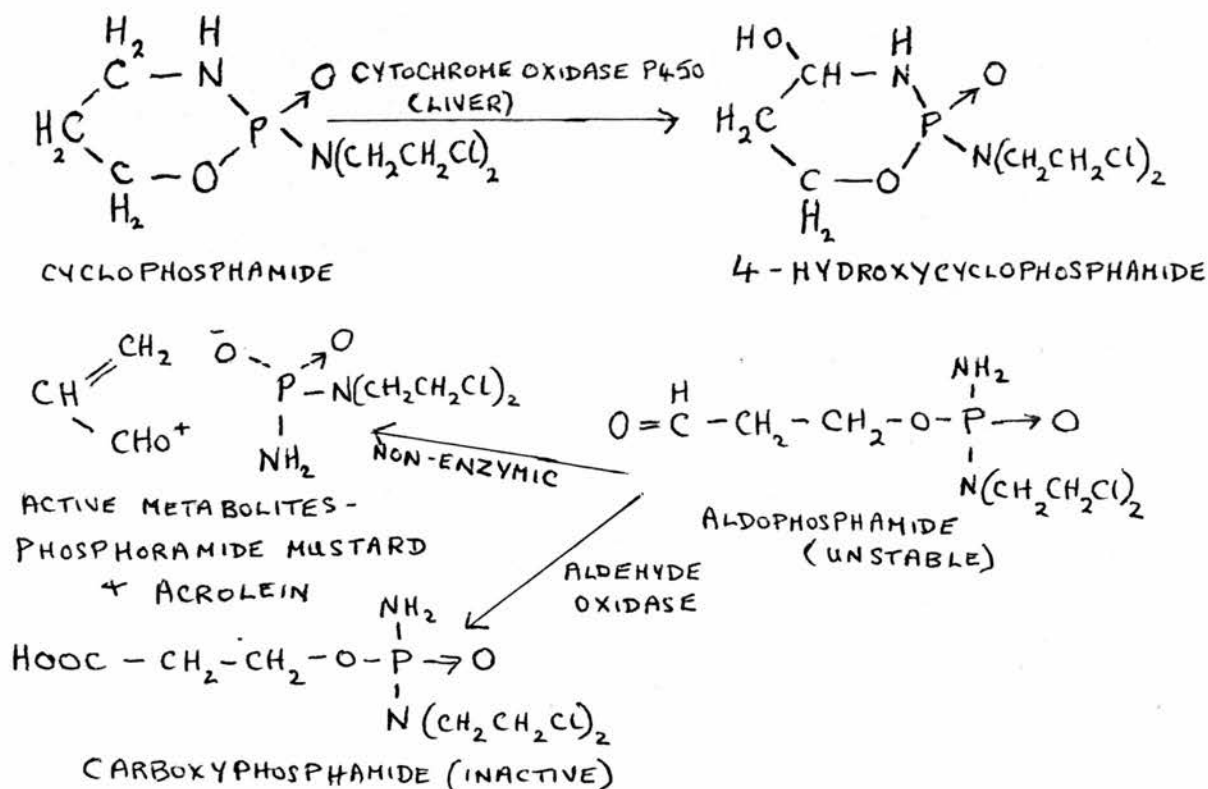
It was first synthesised by the German drug company, Asta-Werke (Arnold, Bourseaux and Brock, 1958).

The alkylating agents act by substituting alkyl ($R - CH_2 - CH_2^+$) groups, usually for the hydrogen ions of organic compounds. When one of these drugs enters a cell, its side chains form covalent bonds with sites on the DNA molecule causing cross-linking and breaks in the molecule. The helix is either prevented from dividing in mitosis, or if it does divide it may reunite in an imperfect form. These effects result in cell death. All rapidly dividing cells are affected and the results resemble damage caused by ionising radiation.

It is a cell cycle non-specific agent, but tends to be more active in S and G₂ phases.

Cyclophosphamide is unusual in that it has little or no activity by itself, and requires activation to an alkylating metabolite by liver microsomes. It was intended to be a transport or "latent" form of the useful but very toxic compound nitrogen mustard, which was the first synthetic compound clearly shown to possess anti-tumour activity in man (Hill, 1975). It was thought that activation would occur selectively in tumours due to the high levels of phosphoramidases present in some tumour tissues, but in fact this does not occur, and organs other than the liver appear to be incapable of activating it.

The metabolic pathway of cyclophosphamide is thought to be qualitatively identical in man and animals, and the following was first proposed by Hill, Laster and Struck (1972):



Carboxyphosphamide and another fairly inactive compound 4-ketocyclophosphamide are the major metabolites excreted in urine and to a small extent in bile, along with unchanged parent drug. The metabolic pathway described above is widely accepted as correct, but there is still considerable controversy about the intermediate metabolites and the exact mechanism of the antitumour effect. Various authors, in a "Symposium on the Metabolism and Mechanism of Action of Cyclophosphamide", discussed this problem, e.g. Donelli, Bartosek, Guaitani, Martini, Colombo, Pacciarini and Modica (1976).

The pharmacology of cyclophosphamide has been studied in many species and was reviewed by Hill (1975). Plasma levels and distribution studies, using radio-labelled cyclophosphamide, have been carried out in the rat, mouse, rabbit, dog, sheep, hamster and monkey, and even in the nurse shark and various other kinds of fish, lobster and protozoa.

In man, there is no binding of the parent drug to plasma proteins, and the plasma disappearance curve shows an initial rapid distribution phase and a second slower phase representing metabolism and excretion according to Bagley, Bostick and DeVita (1973). The half-life of intact plasma cyclophosphamide is 4 to 6.45 hours. These authors also stated that the rate of metabolic activation may be affected by other drugs (e.g. phenobarbital) given at the same time, depending on their effects on liver microsomes, and even by previous doses of cyclophosphamide. The antitumour

and toxic effects are not much affected, however, as they appear to be independent of the rate of metabolic activation, and are related to the dose administered.

Only minute concentrations of the drug are found in the CSF.

Cyclophosphamide is known to have profound effects on the lymphoid system of the body and it is mainly because of this that it has been so successful as an anti-lymphoma agent. The selective depletion of lymphoid tissue in mice and guinea-pigs after dosing with cyclophosphamide was well described in a histological study by Turk and Poulter (1972). They stated that depletion occurred from the lymph follicles, germinal centres, the cortico-medullary junction in lymph nodes and equivalent non thymus-dependent areas of the spleen. After three massive dose injections on alternate days, thymus-dependent areas still contained lymphocytes. Total depletion of lymphocytes could only be produced by neonatal thymectomy or anti-lymphocytic serum treatment along with cyclophosphamide. The animals were killed 3 days after dosing, when the circulating small lymphocyte count in the blood was at its lowest in both species. Histologically the background of reticulum cells was undisturbed, and mature plasma cells in the medullary cords were unaffected. Whorl-like structures of reticulum cells indicated where follicles or germinal centres had been situated.

The mouse thymus showed depletion of lymphocytes

from the cortex only, following a single dose. In the guinea pig, three doses were required for this to occur. Three doses in the mouse caused progressive depletion from the medulla also.

Wagner, Chanana, Cottier, Cronkite, Gassmann, Joel and Laissue (1976) described the effects of two dose levels of cyclophosphamide on the thoracic duct lymphocytes and blood white cells in calves. They decided that there were four phases of effect on thoracic duct lymphocytes:-

- Phase I - initial damage with a decrease in output, especially of large lymphocytes, lasting 8 to 10 hours.
- Phase 2 - an intermediate post-damage recovery phase lasting at least 10 hours.
- Phase 3 - recovery, with overshoot of large thoracic duct lymphocytes and a decrease in output of small thoracic duct lymphocytes (larger dose) and an obvious decrease in blood neutrophil counts, lasting from the 2nd to the 6th day.
- Phase 4 - Stabilization of thoracic duct lymphocyte and blood counts.

They concluded by deducing that "cyclophosphamide may affect the mechanisms controlling the production of B - lymphocytes".

This conclusion was also reached by Anderson, Binns, Symons and Escajadillo (1974) after they injected

cyclophosphamide into 8-day-old piglets and studied lymphoid tissues histologically, indicator cell adherence to lymphoid tissue and surface markers of blood lymphocytes.

However, Milton, Carpenter and Addison (1976) presented evidence that cyclophosphamide also affects T-lymphocyte activity in mice. They found that it depressed various T-cell activities in vitro, such as the proliferative response to phytohaemagglutinin, which fell to about 10% of normal. Skin-graft survival, an in vivo test of T-cell activity, was only mildly affected, and the prolongation of graft survival only occurred if the test was carried out soon after cyclophosphamide treatment.

In conclusion, the authors emphasised the possibility that measurements of T-cell activity may be dependent on the cell origin (e.g. spleen or lymph node) culture conditions, species, and, in vivo, the effects of interactions between T cells, B cells and macrophages.

In man, cyclophosphamide has been widely used in the therapy of neoplastic diseases of many kinds and also in non-neoplastic diseases such as rheumatoid arthritis and nephrotic syndrome in children. It is a powerful immunosuppressant and has been used to prevent rejection of transplanted organs. However, like most anticancer drugs, it has severe toxic side-effects.

The most important side-effect in man is bone marrow suppression, especially of the white cell series.

This was well described by Bergsagel, Robertson and Hasselback (1968) and Buckner, Rudolph, Fefer, Clift, Epstein, Funk, Neiman, Slichter, Storb and Thomas (1972). White cell counts reach a nadir at 9 to 12 days after dosing and then recover during the next 7 days. Platelet counts may also be depressed, though usually only with large doses. Bergsagel et al., (1968) did not find significant changes in blood Hb value with any of the dose levels they used. Other rapidly dividing cells, such as those of the gastro-intestinal mucosa and hair roots, may be affected, therefore vomiting and alopecia are common in humans.

Severe damage to the bladder wall, oedema, necrosis, haemorrhage, hyperplasia and fibrosis, is an important side-effect of cyclophosphamide and its analogues. It is thought to be associated with renal excretion of high concentrations of active metabolites, particularly acrolein, and was first investigated in rats and dogs by Phillips, Sternberg, Cronin and Vidal (1961). This side-effect has caused serious problems for patients and may even be fatal (Spechter, Bauer, Müller and Traut 1965). After methods of preventing cystitis were recognised, recommendations were made that patients should receive a high fluid intake before and during therapy (Leading article British Medical Journal, 1971). Unless very large doses are administered, bladder toxicity is usually avoided if this is carried out. Renal damage has also been reported (Spechter, et al., 1965) and is especially likely if there is pre-existing abnormality of the kidneys.

Severe cardiac toxicity was also reported by Buckner et al. (1972) in patients given high doses of cyclophosphamide. This effect was studied in dogs by O'Connell and Berenbaum (1974), who found that doses equivalent to 250 mg/kg caused severe myocardial damage and pulmonary oedema, and death occurred with a few hours of dosing.

Liver damage appears to be mild, if it occurs at all (Hill, 1975).

Cyclophosphamide is mutagenic, and carcinogenic. It causes sterility, and is teratogenic. Ashby, Davis Penn and Upshall Dewhurst, Espinal (1976) investigated the latter effect in rats. They stated that the teratogenic effect was a function of the whole cyclophosphamide molecule as analogues were not teratogenic even at four times the dose. It was due to the alkylating effect, but, since the embryo was thought to be incapable of converting the drug, they suggested that the effect was mediated through the dam, possibly as a result of altered materno-foetal exchange.

Greenberg and Tanaka (1964) reported human congenital anomalies probably induced by cyclophosphamide.

The haematological and immunological effects of the drug in many species were summarised by Hill (1975).

Biochemical effects of the drug have been recognised in vitro, in animals and in humans (Hill, 1975). Serum enzymes known to be affected in humans and other species are lactate dehydrogenase, alkaline phosphatase and glutamic-oxaloacetic, glutamic-pyruvic and other transaminases, which are elevated, and glucose-6-phosphate

dehydrogenase, malate dehydrogenase and cholinesterase, which are lowered.

The earliest reports of studies using cyclophosphamide were those of Brock and Wilmanns (1958) who investigated the toxicity and therapeutic effect of the drug in rats with Yoshida sarcoma, Jensen sarcoma and Walker carcinoma, and Gross and Lambers (1958a and b) who reported the first clinical trial of cyclophosphamide in humans with various types of cancer. They treated five cases of lymphosarcoma, of which four had a good response and one a partial response, and found the only toxic effects to be slight depression of granulocyte counts, and nausea, loss of appetite and vomiting. Coggins, Ravdin and Eisman (1959) reported on a clinical trial in America in patients with twenty different types of tumour, including nine cases of lymphoma. Eight cases completed therapy and antitumour effect was demonstrated in five of these cases. Side-effects were bone marrow depression, nausea, vomiting, alopecia, (which began about 20 days after the initiation of therapy, with regrowth occurring within 6 to 8 weeks), and sterile cystitis, symptoms of which appeared within 48 hours after a single large dose and lasted 4 to 5 days.

Since then, despite the problems associated with its use, cyclophosphamide has won an undisputed place in cancer chemotherapy. It is administered either orally or, more commonly, intravenously. Several different

dosage schedules have been tried. In the early 1960 s, it was more often administered daily after a loading dose, or as in the trial reported by Carbone, Spurr, Schneiderman, Scotto, Holland and Schnider (1968) in non-Hodgkin's lymphoma cases. The complete remission rate in this study was 11% with a duration of 22 weeks, and a total response rate of 68%. Only 53% of their patients survived for 1 year. Bergsagel, et al., (1968) showed that giving the drug in large intermittent intravenous doses, with 3 or 4 weeks between, was a more successful approach in advanced lung cancer, and Mendelson, Block and Serpick (1970) tried large intermittent doses in lymphoma cases. They reported 5 out of 19 complete remissions (26%), and several partial remissions. Jones, Rosenberg, Kaplan, Kadin and Dorfman (1972) used cyclophosphamide as a single agent in non-Hodgkin's lymphoma, making use of the Rappaport classification, and found that in diffuse poorly-differentiated lymphocytic lymphoma they had a complete remission rate of 22% and a partial remission rate of 45%. O'Connell, Wiernik and Sutherland (1976) published a long-term follow-up of non-Hodgkin's lymphoma patients given high-dose intermittent intravenous cyclophosphamide. There were complete remissions in 5 out of 17 patients, but according to the Rappaport Classification, all of the complete responders had nodular lymphomas. All the diffuse cases showed anti-tumour effect, but died within 20 months. Two of the

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complete responders were "cured" of their lymphomas.

The therapy of the lymphomas was greatly improved by the discovery that combinations of drugs could be used to increase antitumour effect, and cyclophosphamide has become an important component of combined drug schedules for non-Hodgkin's lymphomas. Cyclophosphamide, vincristine and prednisolone have been the most commonly used combination. Early reports of this type of therapy were those by Hoogstraten, Owens, Lenhard, Glidewell, Leone, Olson, Harley, Townsend, Miller and Spurr (1969), Luce, Gamble, Wilson, Monto, Isaacs, Palmer, Coltman, Hewlett, Gehan and Frei (1971) and Bagley, DeVita, Berard and Canellos (1972). Since then, many reports using this and other combinations have been published. There are those who believe that single agent therapy may still have a place in the treatment of non-Hodgkin's lymphomas if the patient has a histological type which has a favourable prognosis (Glatstein, Donaldson, Rosenberg and Kaplan, 1977). However Kennedy, Bloomfield, Kiang, Vosika, Peterson and Theologides (1978) disagreed.

Bearing in mind the information gained from the literature, trials were designed to investigate the effects of cyclophosphamide in normal and lymphosarcomatous pigs.

THE EFFECTS OF CYCLOPHOSPHAMIDE IN NORMAL PIGS

MATERIALS AND METHODS

This was a short-term experiment, extending to only 2 doses per pig, as no cumulative toxicity was expected.

Ten pigs (5 castrated males and 5 females) were used. They were from two litters both sired by the same boar and born on the same day. The sire and both dams were all from non-carrier lines of the lympho-sarcoma strain.

As before, the pigs were allocated by lot to four groups as follows but were housed in a single pen.

Pig No. 407	}	GROUP A	LOW DOSE
Pig No. 415		First dose 20mg/kg, second dose 40mg/kg	
Pig No. 404	}	GROUP B	MEDIUM DOSE
Pig No. 416		First dose 30mg/kg, second dose 60mg/kg	
Pig No. 402	}	GROUP C	HIGH DOSE
Pig No. 412		First dose 40mg/kg, second dose 80mg/kg	
Pig Nos. 405, 409, 411, 414	}	GROUP D	CONTROLS

The control animals were given saline, equal in volume to the medium dose of cyclophosphamide.

At 9 weeks of age, all 10 pigs were sensitised to DNFB, and they were skin-tested a fortnight later (14 days before the first dose). At this time weekly weighing and blood sampling for WBC, RBC, PCV, Hb differential and thrombocyte counts and blood urea

estimation were commenced. On days -7 and 0 serum alkaline phosphatase, total protein and albumin estimations were also carried out. The pigs were observed daily in the pen for appetite, general fitness and state of urine and faeces. A detailed clinical examination, including rectal temperature, condition of visible mucous membranes, and auscultation of heart and lungs, were also carried out on each animal once weekly, from 11 weeks of age.

The preparation used was "Endoxana" (Ward-Blenkinsop, Bracknell). The drug was dissolved in water at a concentration of 20 mg/ml as soon as possible before dosing, as the solution has to be used within 2 hours. The powder dissolved slowly and required shaking.

The drug was administered intravenously on two occasions, 18 days apart. As described in Chapter II the pigs were tranquillised for dosing with 4% azaperone intramuscularly, and the technique of intravenous dosing was as described previously (see page 195). Although the drug was not irritant, tranquillisation was still required because of the large volume of liquid being slowly injected.

During the treatment period, weekly weighing and clinical examinations were continued. The pigs were blood-sampled more frequently. The first dose was

on day 0 and the second on day 18. Complete blood picture, blood serum alkaline phosphatase, and serum total protein and albumin were measured on days 7, 14, 21, and after treatment was stopped, on days 28, 35 and 42. In addition, haematology samples, for WBC counts, differential counts and PCV, were taken on days 1, 2, 3, 4, 5 and 6 after the first dose, and on days 21, 22, 23 and 24 after the second dose. Additional alkaline phosphatase estimations were carried out on serum samples on days 1 and 2.

By recording these results it was hoped to detect evidence of bone marrow depression, urinary tract damage or liver damage, if these toxic side-effects occurred. Different, or more frequent, serum enzyme estimations might have given more complete information but the cost of performing them was prohibitive.

DNFB skin tests were repeated on day 22. Due to unforeseen circumstances, it proved necessary to destroy two of the control pigs before skin tests could be carried out.

When it was considered that effects of the second cyclophosphamide dose would be most marked, two of the four control pigs and one pig from Group A, Group B and Group C were killed by intravenous injection of 20% pentobarbitone. The controls were killed on day 22, the Groups B and C pigs on day 23 and the Group A pig on day 24. All the dead pigs were autopsied within 1 hour.

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The remaining pigs were given no further treatment. Weekly clinical examinations were continued and another skin test was carried out on day 43. By this time, all effects of the drug had apparently disappeared and these pigs were destroyed and autopsied on days 44 and 45.

RESULTS

Clinical Examinations (For clinical records, see Appendix IID).

Throughout the experiment, all the pigs remained fit with excellent appetites. Rectal temperatures stayed within the normal range, below 40°C. Faeces and urine appeared normal, though urine was not sampled for detailed examination. Fighting was a problem in this group of animals, resulting in small cuts, abrasions and contusions. Pigs 402 and 405 developed severe bursitis on the posterior aspect of the tarsi, pig 416 had a bitten tail, pig 415 had a bruised abdomen and a damaged hoof and pig 411 developed cellulitis of the left hind leg and a swollen lower lip which required an intramuscular injection of benzathine penicillin. These injuries occurred before the pigs were dosed with cyclophosphamide as well as after, and there was no evidence that those given cyclophosphamide suffered any more than the control animals. Before the start of dosing, it was observed that pig 407 had a loud heart murmur suggestive of a patent ductus arteriosus. The animal was clinically normal, and although it tended to become dyspnoeic when forced to exercise, it was not inconvenienced by its condition, which did not worsen during the course of the experiment. This animal was one of the pair given the lowest dose level of cyclophosphamide.

The only pig to show any clinical sign which may have been related to cyclophosphamide dosage was one of the high-dose pair, 412. Two weeks after the second dose of the drug (80 mg/kg) the skin and mucous membranes of this animal were noticeably paler than those of the other groups which were left alive. The other pig in this group had already been destroyed by this time.

There was no indication in any of the treated pigs of alopecia or foot lesions.

In a short term experiment such as this it was unlikely that there would be any noticeable effect on weight gains. However the results in Table 36 suggest that growth was retarded in the high-dose group.

TABLE 36

BODY WEIGHT GAINS - CYCLOPHOSPHAMIDE: NORMAL PIGS

Pig No.	Dose level	Wt. at start of treatment (kg)	Wt. at end of treatment (kg)	Gain	Wt. at end of exp. (kg)	Gain	Total Gain
402	40 + 80mg/kg	21	24*	3	-	-	-
412	"	19	24	5	28.5	4.5	9.5
416	30 + 60mg/kg	20	26*	6	-	-	-
404	"	18	25	7	30	5	12
407	20 + 40mg/kg	20.5	27.5*	7	-	-	-
415	"	20.5	29	8.5	36	7	15.5
409	Control	20.5	26.5*	6	-	-	-
411	"	21	26*	5	-	-	-
405	"	18.5	25	6.5	30	5	11.5
414	"	17	29	12	32	3	15

* Destroyed at end of treatment.

TABLE 37

CYCLOPHOSPHAMIDE: NORMAL PIGS

DNFB SKIN-TEST RESULTS:

Groups	Pig Number	At sensi- tisation	Pretreat- ment	End of treatment	3½ weeks after last dose
A - Low dose	407	1	3	3	Dead
	415	1	2	2	3
B - Med. dose	404	1	3	2	2
	416	1	3	1	Dead
C - High dose	402	1	3	1	Dead
	412	1	3	1	3
D - Control	405	1	2	2	1
	409	1	3	Dead	Dead
	411	1	2	Dead	Dead
	414	1	3	4	4

DNFB Skin-Test results

It can be seen from Table 37 that the two control pigs which were tested, and the low-dose pair, had end of treatment reaction scores which were the same as, or greater than, the pretreatment results. However, all four medium and high-dose animals had lower response scores after treatment than pretreatment.

In the test carried out 3½ weeks after the last dose, one control had a slightly reduced response, while the other control's reaction was as severe as on the previous occasion. The low-dose pig had a slightly increased response, the medium-dose pig's reaction was the same as that recorded at the end of treatment, and the high-dose animal had a reaction score which was higher than its end of treatment score, but the same as its pretreatment score.

It would appear, therefore, that treatment with cyclophosphamide temporarily depressed skin hypersensitivity reactions in the medium and high-dose groups.

Haematology

Cyclophosphamide produced a pronounced effect on total WBC counts (See Appendix III). Figure 19 shows the effect clearly, comparing mean counts of the pigs in the high dose group with mean counts of the saline treated controls. Mean total WBC counts and mean absolute counts of total neutrophils, lymphocytes and monocytes \pm ISD are shown in Table 38. Standard

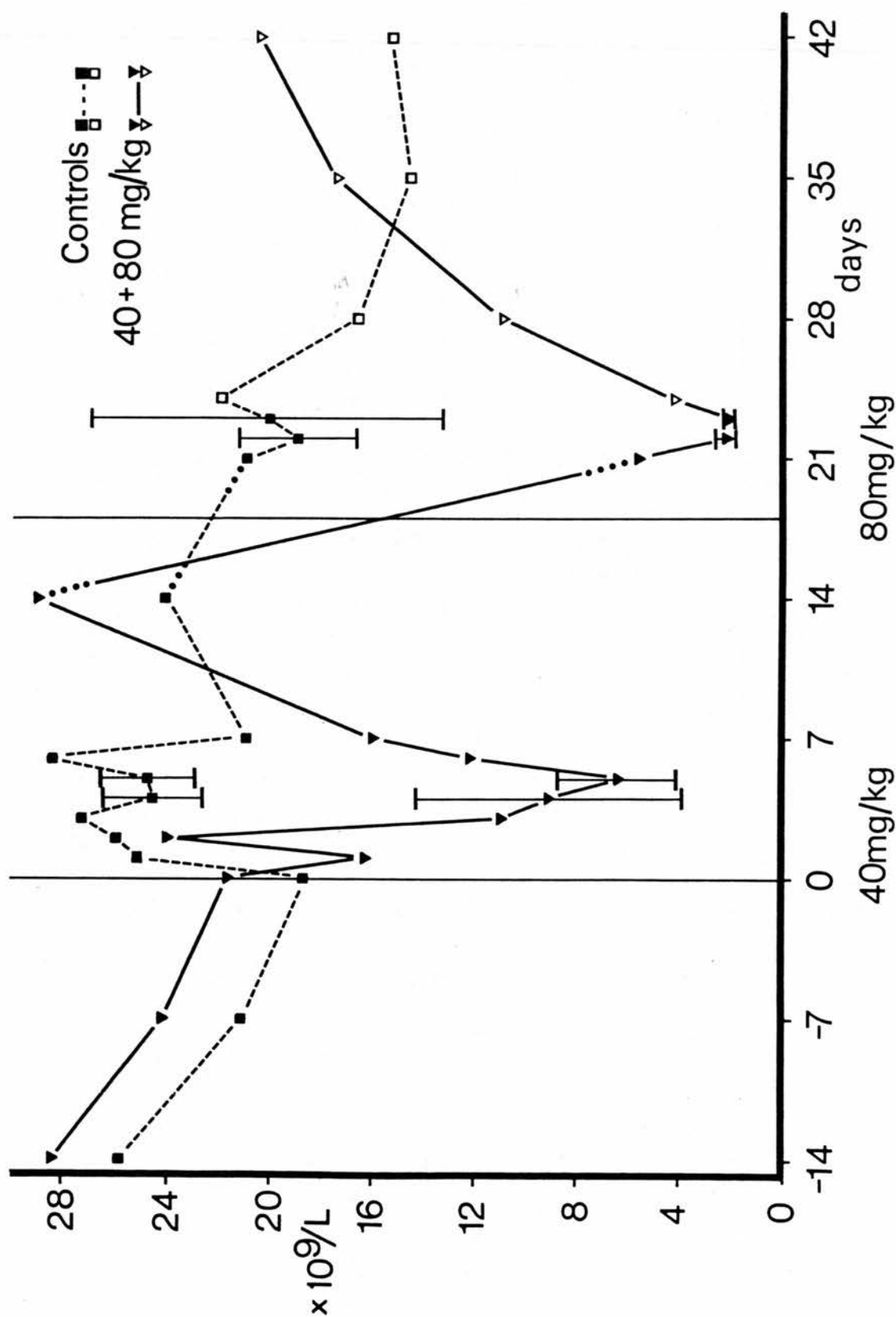


FIGURE 19

Mean WBC counts of two pigs dosed with cyclophosphamide compared with mean counts of four saline-treated controls. Open symbols after half of the pigs were killed.

TABLE 38

CYCLOPHOSPHAMIDE IN NORMAL PIGS										GROUP D - SALINE TREATED CONTROLS				GROUP A - LOW DOSE				GROUP B - MEDIUM DOSE				GROUP C - HIGH DOSE			
DAYS FROM START OF DOSING	WBC - MEANS \pm ISD ($\times 10^9$ /L)				T NEUTROPHILS - MEANS \pm ISD				LYMPHOCYTES - MEANS \pm ISD				MONOCYTES - MEANS \pm ISD												
	GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C					
- 14	25.8 \pm 2.7	26.0 \pm 0.5	25.7 \pm 2.3	28.4 \pm 1.0	10.8 \pm 1.7	15.3 \pm 0.7	12.3 \pm 0.8	12.5 \pm 0.8	13.2 \pm 2.3	8.4 \pm 1.5	11.3 \pm 1.8	14.0 \pm 3.3	1.4 \pm 0.2	2.0 \pm 1.0	1.6 \pm 0.4	1.7 \pm 1.1	1.4 \pm 0.2	2.0 \pm 1.0	1.6 \pm 0.4	1.7 \pm 1.1					
- 7	21.1 \pm 1.5	22.8 \pm 1.1	25.4 \pm 2.8	24.2 \pm 0.9	8.8 \pm 1.3	10.1 \pm 1.8	11.8 \pm 0.1	8.5 \pm 2.4	10.3 \pm 1.3	11.8 \pm 0.4	11.8 \pm 3.1	14.1 \pm 1.3	1.1 \pm 0.4	0.4 \pm 0.3	1.5 \pm 0.2	1.2 \pm 0	1.1 \pm 0.4	0.4 \pm 0.3	1.5 \pm 0.2	1.2 \pm 0					
0	18.7 \pm 1.7	23.1 \pm 5.7	22.2 \pm 0.8	21.7 \pm 5.9	9.1 \pm 2.4	14.3 \pm 7.8	9.6 \pm 2.8	11.9 \pm 6.5	8.0 \pm 1.1	7.9 \pm 2.3	11.4 \pm 1.9	7.3 \pm 2.6	1.1 \pm 0.4	0.5 \pm 0.3	0.9 \pm 0.3	1.8 \pm 1.4	1.1 \pm 0.4	0.5 \pm 0.3	0.9 \pm 0.3	1.8 \pm 1.4					
Dose																									
1 - 0																									
1	25.1 \pm 2.6	24.1 \pm 7.8	21.2 \pm 4.2	16.3 \pm 0.4	10.1 \pm 1.7	12.9 \pm 6.0	9.2 \pm 4.1	8.0 \pm 1.1	12.4 \pm 1.5	9.7 \pm 1.1	11.2 \pm 0.9	7.1 \pm 2.3	1.7 \pm 0.7	0.6 \pm 0.4	0.7 \pm 0.6	0.6 \pm 0.3	1.7 \pm 0.7	0.6 \pm 0.4	0.7 \pm 0.6	0.6 \pm 0.3					
2	25.9 \pm 2.9	19.2 \pm 7.3	25.9 \pm 9.8	24.0 \pm 8.6	11.0 \pm 2.0	9.9 \pm 6.3	17.2 \pm 8.7	16.8 \pm 8.6	13.1 \pm 2.6	7.9 \pm 0.1	8.4 \pm 0.6	6.5 \pm 0.1	1.2 \pm 0.8	1.0 \pm 0.6	0.2 \pm 0.2	0.3 \pm 0.4	1.2 \pm 0.8	1.0 \pm 0.6	0.2 \pm 0.2	0.3 \pm 0.4					
3	27.2 \pm 8.7	16.2 \pm 0.7	18.5 \pm 0	11.0 \pm 3.3	8.8 \pm 3.0	7.7 \pm 0.9	11.3 \pm 3.7	5.2 \pm 3.4	17.1 \pm 11.2	7.8 \pm 0.3	6.4 \pm 3.3	5.6 \pm 0.2	0.8 \pm 0.2	0.5 \pm 0.4	0.5 \pm 0.1	0	0.8 \pm 0.2	0.5 \pm 0.4	0.5 \pm 0.1	0					
4	24.5 \pm 1.9	10.2 \pm 0.6	10.3 \pm 1.5	9.1 \pm 5.2	8.9 \pm 3.2	1.7 \pm 1.1	1.8 \pm 0.6	0.6 \pm 0.8	13.4 \pm 3.1	7.4 \pm 0.4	7.4 \pm 0.3	7.6 \pm 4.5	1.6 \pm 0.6	1.1 \pm 0.1	1.0 \pm 0.5	0.6 \pm 0.2	1.6 \pm 0.6	1.1 \pm 0.1	1.0 \pm 0.5	0.6 \pm 0.2					
5	24.7 \pm 1.8	12.3 \pm 1.5	12.1 \pm 4.9	6.4 \pm 2.3	9.2 \pm 3.7	1.4 \pm 1.3	1.0 \pm 0.8	0.4 \pm 0.6	13.3 \pm 0.3	9.3 \pm 1.7	9.0 \pm 4.2	4.4 \pm 0.1	1.3 \pm 0.5	1.5 \pm 1.0	2.0 \pm 0.1	1.5 \pm 1.5	1.3 \pm 0.5	1.5 \pm 1.0	2.0 \pm 0.1	1.5 \pm 1.5					
6	28.3 \pm 4.3	14.0 \pm 1.7	14.4 \pm 0.2	12.2 \pm 2.8	10.8 \pm 5.2	4.0 \pm 1.5	3.5 \pm 0	1.8 \pm 0.9	15.5 \pm 5.3	9.1 \pm 0	8.6 \pm 0.3	8.2 \pm 0.9	1.1 \pm 0.4	0.9 \pm 0.2	2.2 \pm 0.5	2.3 \pm 1.0	1.1 \pm 0.4	0.9 \pm 0.2	2.2 \pm 0.5	2.3 \pm 1.0					
7	20.9 \pm 1.2	16.4 \pm 1.5	16.8 \pm 0.3	16.0 \pm 1.3	8.3 \pm 1.4	6.6 \pm 1.5	5.8 \pm 0.5	4.7 \pm 1.2	11.0 \pm 0.9	8.6 \pm 0.3	8.3 \pm 0.7	9.1 \pm 1.4	1.4 \pm 0.3	1.1 \pm 0.4	2.5 \pm 0.8	1.9 \pm 1.3	1.4 \pm 0.3	1.1 \pm 0.4	2.5 \pm 0.8	1.9 \pm 1.3					
14	24.0 \pm 1.5	25.0 \pm 0.9	33.8 \pm 2.5	28.9 \pm 0.6	10.6 \pm 1.7	13.4 \pm 1.4	18.9 \pm 1.4	13.6 \pm 1.9	11.4 \pm 0.7	10.8 \pm 0.5	13.8 \pm 1.0	13.5 \pm 1.7	0.8 \pm 0.1	0.7 \pm 0	1.0 \pm 0.1	1.3 \pm 0.6	0.8 \pm 0.1	0.7 \pm 0	1.0 \pm 0.1	1.3 \pm 0.6					
Dose																									
2 - 18																									
21	20.9 \pm 1.9	12.7 \pm 3.5	11.1 \pm 1.3	5.6 \pm 2.5	7.8 \pm 2.1	8.6 \pm 2.8	8.6 \pm 1.4	3.4 \pm 2.6	10.3 \pm 2.1	3.7 \pm 0.5	2.4 \pm 0.2	1.7 \pm 0.1	1.6 \pm 0.8	0.3 \pm 0.1	0	0.2 \pm 0.1	1.6 \pm 0.8	0.3 \pm 0.1	0	0.2 \pm 0.1					
22	18.9 \pm 2.3	5.6 \pm 1.1	3.5 \pm 0	2.2 \pm 0.4	6.8 \pm 0.7	1.3 \pm 0.8	0.4 \pm 0	0.1 \pm 0.1	10.4 \pm 1.7	3.9 \pm 0.1	2.9 \pm 0.1	2.0 \pm 0.3	0.9 \pm 0.2	0.3 \pm 0.1	0.1 \pm 0.1	0	0.9 \pm 0.2	0.3 \pm 0.1	0.1 \pm 0.1	0					
23	20.0 \pm 6.8	4.6 \pm 0.5	2.6 \pm 0.4	2.1 \pm 0.1	9.5 \pm 5.1	0.1 \pm 0	0.1 \pm 0.1	0.1 \pm 0.1	8.9 \pm 2.1	4.0 \pm 0.5	2.2 \pm 0.2	1.8 \pm 0.2	1.2 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.1	0.2 \pm 0.2	1.2 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.1	0.2 \pm 0.2					
24	21.9 \pm 5.9	8.4 \pm 0.4	5.1	4.1	10.6 \pm 4.5	0.5 \pm 0.2	0.6	1.0	9.9 \pm 1.5	5.3 \pm 0.9	3.2	1.8	1.0 \pm 0.3	2.4 \pm 1.5	1.4	1.3	1.0 \pm 0.3	2.4 \pm 1.5	1.4	1.3					
28	16.6 \pm 5.4	17.6	12.6	10.9	6.1 \pm 1.5	11.1	5.2	5.1	9.0 \pm 4.3	5.5	6.3	4.9	1.1 \pm 0.1	1.1	1.1	0.5	1.1 \pm 0.1	1.1	1.1	0.5					
35	14.5 \pm 1.9	19.5	20.9	17.4	4.8 \pm 0.5	9.8	13.6	10.1	8.1 \pm 1.5	7.4	6.3	5.2	1.3 \pm 0.8	1.4	0.8	1.6	1.3 \pm 0.8	1.4	0.8	1.6					
42	15.2 \pm 1.1	19.6	16.0	20.4	6.9 \pm 2.4	11.4	8.8	11.8	7.0 \pm 1.6	7.1	6.2	6.1	0.9 \pm 0.1	0.4	0.5	1.6	0.9 \pm 0.1	0.4	0.5	1.6					

deviations are shown on the graph where they do not confuse the picture, otherwise see Table 38.

In all the cyclophosphamide-treated animals there was a depression of WBC counts after each dose, the lowest counts being obtained on the 4th or 5th day after dosing. There was a rapid recovery, and by 14 days after dosing, the counts of the treated pigs were similar to, or higher than, the mean counts of the controls. Table 38 shows that the three types of white cell were all affected, but neutrophils were most severely depressed. At 5 days after dosing, pigs which were given 40mg/kg, or more, had so few neutrophils, that it became very difficult to find any at all on the blood smear when performing a differential count. Neutrophil counts in Group A and B pigs, given 20 and 30 mg/kg, were not quite so severely depressed, but at 5 days the counts were too low to give any clear indication of a dose-related effect.

When the mean lymphocyte counts are examined, the effects are more complex. After the first dose, lymphocyte counts in all pigs were depressed but not to the same extent as the neutrophils. The nadir in the low-dose pigs was at 4 days, in the medium-dose pigs at 3 days and in the high-dose pigs at 5 days, although the latter group had low lymphocyte counts on day 3 also. After the second dose, in the low-dose animals, the nadir in the lymphocyte counts was 3 days later, though the counts at 4 and 5 days were also low. In the medium-dose pigs, the mean counts 3 and 5 days

after dosing were lower than at 4 days with the nadir at 5 days, and in the pigs which received the highest dose of all, the same pattern occurred with the nadir at 3 days. The slight increase in the counts at 4 days after dosing is also apparent when the figures for individual pigs are examined. (See Appendix III). The effect of cyclophosphamide on the lymphocytes was clearly dose-related, the lowest counts being recorded in the pigs receiving the highest dose (Table 38).

Mean monocyte counts in the treated pigs also appeared to be depressed, particularly on the 3rd day after dosing, and from the 5th to the 7th day, counts higher than those of the controls were obtained. However because of the small numbers of monocytes counted normally, it was difficult to assess the meaning of the latter observation.

Mean values of RBC counts, Hb, PCV and MCV (See Appendix III for individual results) in the high-dose group compared with those of the controls are shown in Figure 20A and 20B. Mean values \pm ISD of RBC counts, Hb, PCV, MCV, MCHC and MCH in all groups are shown in Table 39. In all the treated groups, cyclophosphamide, caused a depression in RBC counts, PCV and Hb, though in the low-dose group it was very mild. Only PCV was measured daily after dosing and these figures indicate that the maximum depression occurred at 6 days after both the first and second doses, apart from the medium-dose pig which was not destroyed. It had a low

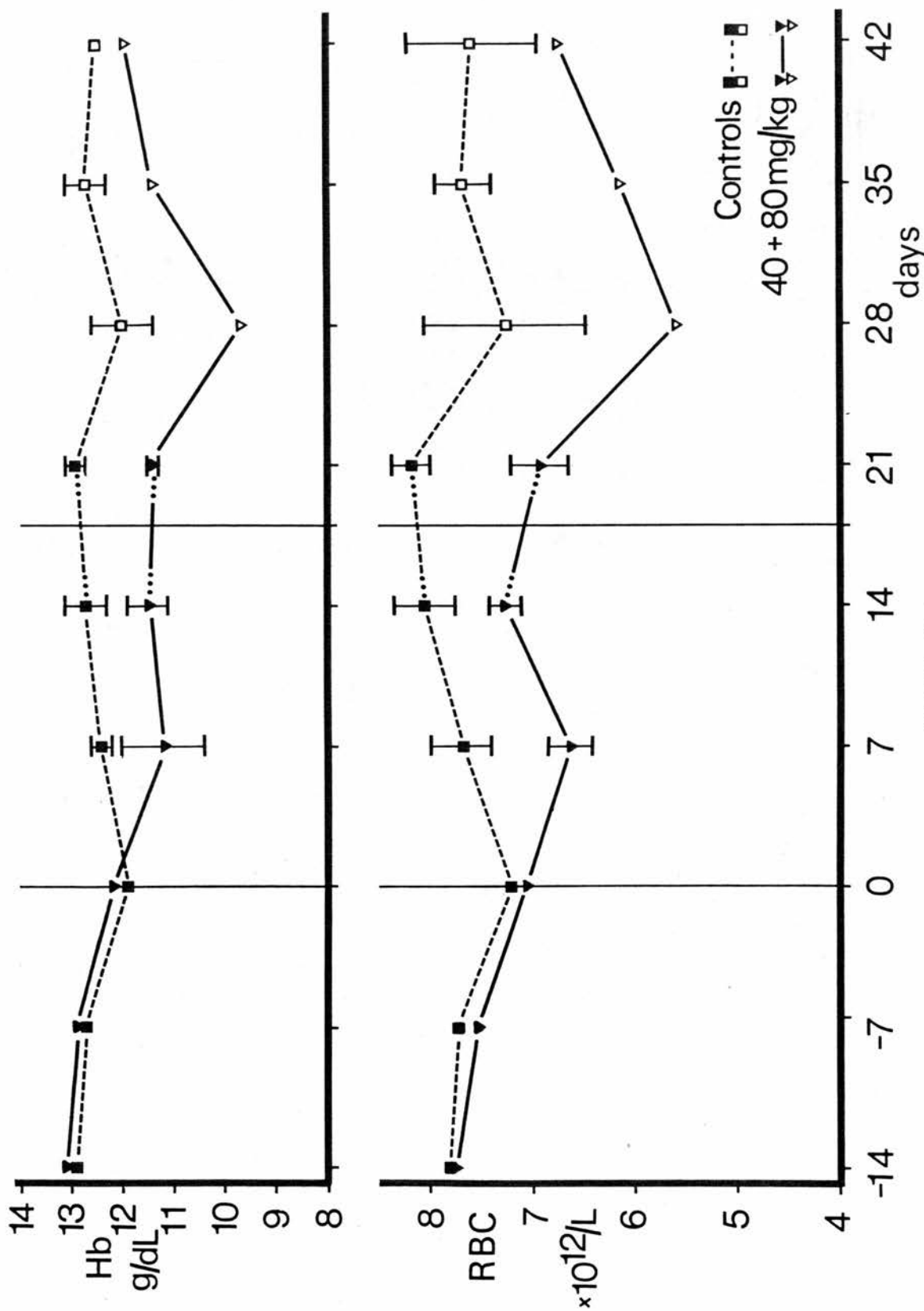


FIGURE 20A

Mean Hb and RBC counts of two pigs dosed with cyclophosphamide compared with results of four saline-treated controls. Open symbols after half of the pigs killed. Vertical lines indicate times of dosing.

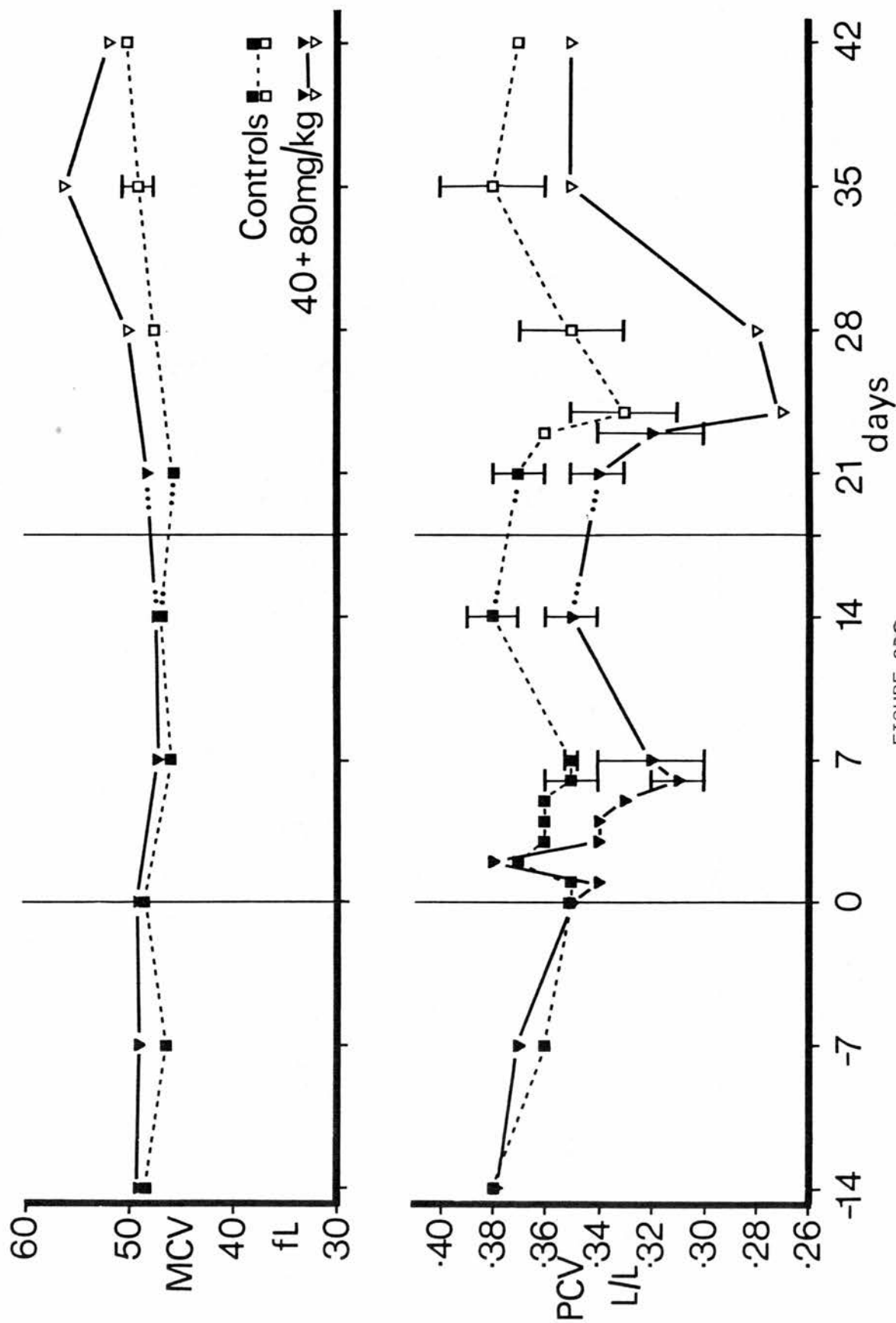


FIGURE 208

Mean PCV and MCV of two pigs dosed with cyclophosphamide compared with results of four saline-treated controls. Open symbols after half of the pigs were killed. Vertical lines indicate times of dosing.

TABLE 39

CYCLOPHOSPHAMIDE IN NORMAL PIGS

GROUP D - SALINE - TREATED CONTROLS

GROUP A - LOW DOSE

GROUP B - MEDIUM DOSE

GROUP C - HIGH DOSE

DAYS

FROM

START

OF

DOSING

RBC - MEANS \pm I S D ($\times 10^{12}/l$)PCV MEANS \pm I S D (1/1)HB CONCENTRATION - MEANS \pm I S D (g/dl)

	GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C	GROUP D	GROUP A	GROUP B	GROUP C
- 14	7.81 \pm 0.12	7.68 \pm 0.35	6.82 \pm 0.30	7.79 \pm 0.28	0.38 \pm 0.01	0.37 \pm 0.01	0.36 \pm 0.01	0.38 \pm 0	12.9 \pm 0.4	12.6 \pm 0	11.9 \pm 0	13.1 \pm 0.1
- 7	7.72 \pm 0.37	7.68 \pm 0.43	7.38 \pm 0.44	7.54 \pm 0.46	0.36 \pm 0.01	0.36 \pm 0.02	0.36 \pm 0.01	0.37 \pm 0.02	12.7 \pm 0.4	12.6 \pm 0.2	12.4 \pm 0	12.9 \pm 0.9
0	7.22 \pm 0.08	7.25 \pm 0.49	7.13 \pm 0.30	7.07 \pm 0.15	0.35 \pm 0	0.35 \pm 0	0.34 \pm 0.01	0.35 \pm 0	11.9 \pm 0.5	12.1 \pm 0.1	12.0 \pm 0.4	12.2 \pm 0.1
Dose												
1 - 0												
1					0.35 \pm 0	0.35 \pm 0	0.35 \pm 0.01	0.34 \pm 0				
2					0.37 \pm 0.01	0.37 \pm 0.01	0.36 \pm 0.01	0.38 \pm 0.02				
3					0.36 \pm 0.01	0.34 \pm 0.03	0.35 \pm 0.01	0.34 \pm 0				
4					0.36 \pm 0.02	0.36 \pm 0.01	0.33 \pm 0	0.34 \pm 0.01				
5					0.36 \pm 0.01	0.33 \pm 0.01	0.32 \pm 0.01	0.33 \pm 0				
6					0.35 \pm 0.01	0.32 \pm 0.01	0.32 \pm 0	0.31 \pm 0.01				
7	7.69 \pm 0.30	7.35 \pm 0.83	6.98 \pm 0.26	6.64 \pm 0.22	0.35 \pm 0	0.35 \pm 0.03	0.33 \pm 0.01	0.32 \pm 0.02	12.4 \pm 0.2	11.9 \pm 0.7	11.3 \pm 0.1	11.2 \pm 0.8
14	8.05 \pm 0.29	7.48 \pm 0.66	7.45 \pm 0.34	7.28 \pm 0.15	0.38 \pm 0.01	0.36 \pm 0.04	0.35 \pm 0.02	0.35 \pm 0.01	12.7 \pm 0.4	12.0 \pm 1.1	12.2 \pm 0.7	11.5 \pm 0.4
Dose												
2 - 18												
21	8.18 \pm 0.19	7.10 \pm 0.67	7.10 \pm 0.50	6.94 \pm 0.28	0.37 \pm 0.01	0.34 \pm 0.02	0.34 \pm 0.01	0.34 \pm 0.01	12.9 \pm 0.2	11.5 \pm 0.6	11.3 \pm 0.1	11.4 \pm 0.1
22												
23 +					0.36 \pm 0	0.32 \pm 0.03	0.31 \pm 0.01	0.32 \pm 0.02				
24 +					0.33 \pm 0.02	0.32 \pm 0.02	0.37	0.27				
28	7.27 \pm 0.80	7.24	6.18	5.58	0.35 \pm 0.02	0.33	0.29	0.28	12.0 \pm 0.6	11.5	9.9	9.7
35	7.69 \pm 0.27	7.32	6.99	6.14	0.38 \pm 0.02	0.36	0.35	0.35	12.7 \pm 0.4	12.3	11.5	11.4
42	7.60 \pm 0.64	7.87	6.86	6.75	0.37 \pm 0.02	0.38	0.33	0.35	12.5 \pm 0.6	12.9	11.5	11.9
CYCLOPHOSPHAMIDE												
EXPERIMENT												
MCV - MEANS \pm I S D												
- 14	48.5 \pm 1.7	47.9 \pm 0.8	52.9 \pm 3.4	49.2 \pm 2.2	33.9 \pm 0.5	34.2 \pm 0.8	32.9 \pm 0.8	34.3 \pm 0.6	16.5 \pm 0.7	16.4 \pm 0.6	17.4 \pm 0.7	16.8 \pm 0.4
- 7	46.5 \pm 1.3	47.5 \pm 0	48.2 \pm 1.9	49.1 \pm 0.1	35.3 \pm 0.7	34.7 \pm 1.1	34.9 \pm 0.7	34.8 \pm 0.5	16.4 \pm 0.8	16.5 \pm 0.5	16.8 \pm 1.0	17.1 \pm 0.2
0	48.5 \pm 0.5	48.0 \pm 2.8	47.8 \pm 1.1	49.2 \pm 0.6	33.9 \pm 1.5	34.7 \pm 0.1	35.2 \pm 0.4	35.0 \pm 0.1	16.5 \pm 0.7	16.7 \pm 1.1	16.8 \pm 0.2	17.2 \pm 0.3
Dose												
1 - 0												
7	45.9 \pm 1.9	47.4 \pm 2.3	46.9 \pm 0.3	47.4 \pm 1.6	35.2 \pm 0.3	34.3 \pm 0.4	34.5 \pm 0.7	35.6 \pm 0.4	16.2 \pm 0.8	16.2 \pm 1.0	16.2 \pm 0.4	16.9 \pm 0.7
14	46.9 \pm 2.4	47.5 \pm 0.5	46.7 \pm 0.2	47.4 \pm 0.3	33.6 \pm 1.0	33.7 \pm 0.4	35.1 \pm 0.3	33.3 \pm 0.6	15.7 \pm 0.6	16.0 \pm 0	16.4 \pm 0.2	15.8 \pm 0.1
Dose												
2 - 18												
21	45.7 \pm 1.2	47.3 \pm 1.5	47.3 \pm 2.4	48.3 \pm 1.1	34.6 \pm 0.9	34.2 \pm 0.3	33.7 \pm 0.3	33.9 \pm 0.5	15.8 \pm 0.3	16.2 \pm 0.6	16.0 \pm 0.9	16.4 \pm 0.6
28 +	47.6 \pm 2.4	45.6	46.9	50.2	34.8 \pm 0.6	34.8	34.1	34.6	16.6 \pm 1.1	15.9	16.0	17.4
35	49.1 \pm 1.4	49.2	49.4	56.2	33.7 \pm 1.1	34.2	33.3	33.0	16.6 \pm 0.1	16.8	16.5	18.6
42	50.2 \pm 4.3	48.3	48.1	51.9	33.9 \pm 0	33.9	34.8	34.0	17.1 \pm 1.5	16.4	16.8	17.6

CYCLOPHOSPHAMIDE

EXPERIMENT

MCV - MEANS \pm I S DMCHC - MEANS \pm I S DMCH - MEANS \pm I S D

TABLE 39 Contd.

PCV at 10 days after the second dose. The only pig to show clinical anaemia was the high-dose animal 412, during the 2nd week after the 80 mg/kg dose. The figures from this animal on day 28 (See Appendix III Table 29) show that this observation was valid but the anaemia was not severe and recovery was rapid.

Mean MCHC values in all groups were similar to those of the controls and only the high-dose pig 412 showed a change in MCV and MCH. This was a marked increase which was calculated from the figures obtained on day 35, 17 days after the second dose.

Mean thrombocyte counts \pm 1 S.D. in the treated animals and in the controls are shown in Table 40 . Counts in the treated animals, especially those in the high-dose pair, were slightly lower than counts in the control group, at 7 days after the first dose and at 7 and 10 days after the second dose, but none of the treated animals was ever dangerously thrombocytopenic.

TABLE 40

CYCLOPHOSPHAMIDE: NORMAL PIGS - MEAN THROMBOCYTE COUNTS \pm 1 S.D. $\times 10^9/L$.

Days from start Of Dosing	Group D - Controls	Group A - Low	Group B - Medium	Group C - High
- 14	362 \pm 42	280 \pm 24	335 \pm 57	310 \pm 10
- 7	367 \pm 37	484 \pm 236	367 \pm 47	266 \pm 27
0	296 \pm 67	274 \pm 1	331 \pm 49	235 \pm 28
Dose 1 - 0				
7	331 \pm 80	227 \pm 7	250 \pm 37	181 \pm 98
14	316 \pm 41	283 \pm 16	361 \pm 68	366 \pm 20
Dose 2 - 18				
21	354 \pm 27	239 \pm 37	303 \pm 69	225 \pm 33
28*	325 \pm 25	213	175	262
35*	286 \pm 29	335	340	360
42*	219 \pm 25	219	305	310

* Two control pigs and one pig in each of the treated groups.

Serum Alkaline Phosphatase

See Table 31 Appendix III for values in individual animals.

There was considerable variation in the levels of S.A.P. between animals, but all figures obtained were within the normal range or slightly below normal.

One animal in each treated group, numbers 402, 404 and 407, showed a slight rise 1 day after the first dose of cyclophosphamide. Pig 404, which received a medium dose had another slight increase on day 2. Low levels were recorded 3 days after the second dose, but levels in the treated groups were similar to those in the control group.

Blood Urea (See Appendix III Tables 21 to 30)

Blood urea measurements in the treated pigs showed no differences from those in the control pigs, which could be attributed to the treatment. All urea results, before, during and after treatment, were within the normal range.

Serum Proteins (See Appendix III Table 32)

Total protein and albumin were measured from serum as previously described, and total serum globulin levels were calculated. Means \pm 1 S.D. of albumin and globulins are shown in Table 41.

Serum albumin rose in all the groups on day 7 and fell again by day 14. There was a pronounced decrease in the high-dose group (C), on day 14. There was a further decrease in all groups after the second

TABLE 41

CYCLOPHOSPHAMIDE: NORMAL PIGS

SERUM PROTEINS - MEAN \pm 1 S.D. (g/l)

Days From Start Of Dosing	SERUM ALBUMIN			SERUM GLOBULINS (BY DIFFERENCE)				
	Group D	Group A	Group B	Group C	Group D	Group A	Group B	Group C
- 7	31.0 ^{+1.6}	30.4 ^{+4.7}	34.2 ^{+3.4}	33.0 ^{+2.4}	29.0 ^{+1.8}	25.9 ^{+2.8}	27.6 ^{+0.8}	26.5 ^{+1.1}
0	28.9 ^{+1.9}	29.8 ^{+2.6}	31.6 ^{+0.6}	31.9 ^{+4.0}	28.6 ^{+3.1}	27.2 ^{+0.1}	26.8 ^{+1.1}	26.7 ^{+3.5}
Dose 1-0								
7	32.3 ^{+5.4}	35.2 ^{+0.1}	36.6 ^{+0.4}	32.8 ^{+2.3}	29.3 ^{+6.8}	28.9 ^{+4.7}	26.8 ^{+3.3}	30.2 ^{+1.2}
14	31.6 ^{+2.2}	29.7 ^{+1.8}	34.2 ^{+0.6}	25.9 ^{+1.6}	25.7 ^{+0.7}	24.0 ^{+1.6}	25.0 ^{+0.8}	34.4 ^{+1.7}
Dose 2-18								
21	26.7 ^{+2.0}	22.0 ^{+0.2}	20.6 ^{+6.2}	20.0 ^{+3.3}	35.0 ^{+5.4}	29.4 ^{+2.9}	38.9 ^{+6.7}	37.2 ^{+3.9}
28*	28.1 ^{+2.5}	22.1	26.6	24.6	29.5 ^{+3.4}	28.1	24.3	27.0
35*	27.3 ^{+0.1}	24.3	24.0	22.1	33.5 ^{+3.1}	29.7	32.6	35.4
42*	27.4 ^{+1.1}	25.9	23.3	21.9	28.6 ^{+6.2}	30.0	29.9	29.3

* Two Control Pigs and One Pig in Each of the Treated Groups.

dose on day 21. This also happened in the control animals and so it may not have been related to the treatment.

There was a rise in serum globulins in the high-dose group on day 14, corresponding to the fall in albumin, but otherwise there were no differences in globulins between groups which could be attributed to the treatment. There was an increase in globulins in all groups on day 21.

Post-Mortem Findings

409 - Control

The carcass and viscera of this animal were normal. The spleen was small and the cut surface exuded blood 1 mm white pulp nodules visible.

The thymus was large with pale fawn soft lobules.

The prescapular lymph nodes were fawn in colour.

The inguinal nodes showed half-and-half pale fawn cortex and darker medulla, as did the popliteals.

The iliac lymph nodes had 25% cortex and 75% medulla.

The mesenteric nodes showed 5 mm white nodular cortex surrounded by 1 mm fawn or red medulla.

The gastrics had 5 mm white cortex surrounded by 1 mm pale fawn medulla.

Other lymph nodes showed pale fawn colouration with an ill-defined slightly darker medulla.

411 - Control

This animal was normal in carcass and viscera.

The spleen was of moderate size and on cut surface showed 1 mm white pulp nodules in a red engorged background.

The thymus was large and composed of pale soft lobules.

The bronchial lymph nodes were large with 4 mm white cortical nodules and 4 mm pink medulla.

The iliacs and inguinals showed a dark brown 1 mm medulla around 3 mm white cortical nodules.

All other lymph nodes showed 3 mm white nodular cortex surrounded by 1 mm fawn medulla.

405 - Control

This animal was normal in carcass and viscera.

The bone marrow at the ends of the fat in the shaft of the femur showed slight red activity. It was similar to 414 (See below).

414 - Control

The carcass and viscera of this animal were normal.

The femoral bone marrow showed slight red activity at the ends of the fat in the shaft.

The popliteal, inguinal and iliac lymph nodes showed dark brown medulla, and the prescapular showed some haemorrhage and some white nodules, possibly associated with blood-sampling shortly before death.

407 - Low Dose

This was an anatomically normal carcass except for the heart. The ductus arteriosus

was 4 mm in diameter, short in length with roughened lining but there was no blood coagulum filling it. The foramen ovale showed a 1 cm diameter patency and the valve flaps did not completely cover it. The lungs were normal. The liver showed a slight diffuse pallor but lobulation and consistency were normal.

The spleen was small and on cut surface was red with blood but no white pulp was visible.

The bladder mucous membrane was slightly nodular and oedematous, with small petechiae scattered all over it.

The thymus lobules were large, pale and soft.

All lymph nodes showed 3 to 4 mm white cortical nodules surrounded by 1 to 2 mm pink/brown medulla, except for the popliteals which were mainly white with only a trace of brown, the iliacs which showed half and half white cortex and dark brown medulla, and the inguinals mesenterics, gastrics, prescapular and bronchials which had 2 to 3 mm white cortical nodules with 1 mm brown medulla.

The bone marrow of the femur was normal.

415 - Low Dose

The carcass and viscera of this animal were normal.

The bladder showed no abnormality.

The spleen showed much blood on cut surface, which was red with no white pulp visible.

The thymus was composed of fawn large lobules, fairly firm in consistency.

All lymph nodes showed 3 to 4 mm white cortex surrounded by a hint of medulla, which was slightly wider and darker in colour in the popliteal, iliac and inguinal lymph nodes.

The bone marrow of the femur showed fat throughout the shaft.

416 - Medium Dose

This was a well-developed, well-nourished anatomically normal carcase.

The liver showed a slight diffuse pallor but the consistency and lobulation were normal.

The bladder was full but normal.

The spleen was small and the cut surface was red, showing no visible white pulp.

The thymus showed moderate-sized lobules which were soft and pale fawn/white in colour.

All lymph nodes showed half-and-half fawn medulla and white cortex, except for the bronchials which showed 1 mm white nodules surrounded by 3 mm red medulla, the iliacs which had 1 mm white cortex surrounded by 2 mm fawn/brown medulla, the inguinals and popliteals which had 3 to 4 mm white cortex surrounded by 1 to 2 mm dark brown medulla, the mesenterics and colonics which showed 1 to 2 mm white cortex on a pink/buff background, and the gastrics which showed 2 mm white nodules with 1 mm fawn medulla.

The bone marrow of the femur was normal.

404 - Medium Dose

This was an anatomically normal carcass, similar to 412.

The iliac, inguinal and popliteal lymph nodes showed a 1 mm dark brown medulla.

The femoral marrow showed a little pink activity at each end of the fat in the shaft.

402 - High Dose

This was a well-developed, well-nourished carcass and was anatomically normal.

The liver showed a slight diffuse pallor but there was no change in consistency or degree of lobulation.

The bladder was full but normal.

The spleen was small with no white pulp visible on cut surface.

The thymus was white and soft in consistency and the lobules were small.

All lymph nodes showed white trabeculae surrounded by 1 to 3 mm pink or white cortical nodules surrounded by a 1 to 2 mm pink, fawn or brown medulla.

The bone marrow of the femur was fatty.

412 - High Dose

This was an anatomically normal carcass.

The pancreas was slightly browner than normal.

The spleen was red and on cut surface showed no visible white pulp. The lateral border was folded backwards onto the non-hilar surface but was not adherent to it.

The bladder was normal.

The ovaries showed many small Graafian follicles but the uterus was small and inactive.

The thymus was of moderate size, the lobules pale but slightly firmer than normal.

All carcass lymph nodes showed white/fawn 3 to 4 mm cortical nodules with a hint of darker fawn medulla round about.

The marrow of the femur was fatty throughout the whole of its length.

Lymph Node and Organ Weights

See Appendix III for absolute weights in grams. Table 42 shows the effects of cyclophosphamide on relative weights of lymph nodes and organs (cg/kg). At the end of the treatment period, the most noticeable effect was a moderate decrease in the weights of the thymuses of the treated pigs (mean of 90.6 ± 4.2 compared with 142 ± 41 in the two control pigs). Lymph node weights in the treated pigs were only marginally less than in the controls.

At the end of the experiment, the thymus in the low dose pig (415) was involuted, but the thymuses of the other two treated pigs appeared to be much larger than those of the controls. The mean thymus weight in all three treated pigs was 153 ± 76.6 compared with 119.5 ± 24.7 in the controls, but the high and medium dose pigs had relative thymus weights of 221 and 168. Lymph node and other organ weights in the treated pigs were similar to those of the controls.

TABLE 42

CYCLOPHOSPHAMIDE : NORMAL PIGS - RELATIVE WEIGHTS OF LYMPH NODES AND ORGANS (cg/kg BODY WEIGHT)

DOSE LEVEL	PIGS KILLED AT END OF TREATMENT			PIGS KILLED AT END OF EXPERIMENT		
	CONTROLS	LOW	MEDIUM HIGH	CONTROLS	LOW	MEDIUM HIGH
PIG NUMBER	409 411	407	416 402	405 414	415	404 412
LYMPH NODES :						
Mesenteric and Colonic	377 327	291	385 333	300 313	278	373 318
Gastric and Splenic	55 43	25	29 41	31 48	27	27 42
Bronchial Group	16 30	14	20 10	10 11	8	10 15
Total Splanchnic	448 400	330	434 384	341 372	313	410 375
Head Group	32 34	37	27 26	28 27	30	28 41
Iliac Group	6 7	8	5 8	9 9	7	7 9
Prescapular	2 2	7	5 4	4 2	4	3 6
Precrural	4 5	3	2 4	3 3	3	3 3
Inguinal	11 17	11	8 14	10 10	11	12 11
Popliteal	3 2	1	1 1	2 3	2	1 1
Total Superficial	58 67	67	48 57	56 54	57	54 71
Thymus	113 171	94	92 86	102 137	70	168 221
Spleen	283 327	182	346 375	300 219	194	283 351
Liver	3925 3788	3636	3327 3438	2500 2906	2306	3133 3474
Kidney	226 288	262	269 258	183 195	167	217 211
Adrenal	6 6	7	6 8	5 6	4	5 6

DISCUSSION

The results of this experiment were surprising because of the lack of severe toxicity compared with the effects of the drug in man (See Introduction to this chapter), and those described by Lee, Castles and Kintner (1973). Their trial was one of the most relevant to the use of the drug in man and investigated the toxicity of various single doses of cyclophosphamide in dogs and monkeys. Dogs were more severely affected by the drug than monkeys. In the dog, single intravenous doses of 2.5 mg/kg were non-toxic, apart from mild haematological changes. A dose of 20 mg/kg caused serious changes and lesions, and 40 mg/kg was lethal. Toxic signs were emesis, bloody diarrhoea, haematuria, excessive salivation, poor appetite or anorexia and weight loss, with leucopenia, thrombocytopenia, reticulocytopenia, decreases in haematocrit and haemoglobin values and changes in blood chemistry. At autopsy, lesions included degenerative and inflammatory changes in the intestinal tract, haemorrhagic cystitis, lymphoid tissue destruction and bone marrow depression. In the monkey, 2.5 mg/kg caused mild haematological changes only, but 80 mg/kg, though it caused serious effects, was not lethal. Similar changes to those observed in dogs occurred but were less severe. In addition, the monkeys exhibited alopecia. Cystitis occurred in two monkeys, one of

which was given only 10 mg/kg. Leucopenia occurred in both species within 1 week of dosing. Relative organ weights of treated and control animals were given, but lymph node weights were not included.

In the treated pigs, the effects observed were a decrease in weight gain at the highest dose level, a temporary depression of skin hypersensitivity reactions in the medium and high dose groups, a fall in white cell counts at from 3 to 5 days after dosing, with a particularly severe but temporary depression of neutrophil counts, a fall in red cell parameters at 6 days after dosing and a very slight reduction in thrombocyte counts at 7 to 10 days.

The haematological effects were very similar to those which have been recorded in other animals and in man (Hill, 1975), though the nadir of WBC counts appears to occur rather later in man at 9 to 12 days after dosing (Bergsagel et al., 1968). Depression of red cell parameters and thrombocyte counts do not appear to be important features of the toxicity of cyclophosphamide in man, however. The fact that cyclophosphamide causes alopecia may prove to be useful in the sheep. The wool can be stripped off by hand 7 to 14 days after a carefully regulated dose (Homan, Zendzian, Busey and Rall, 1969).

However, in the pigs there was no evidence of alopecia or hoof changes. These might not have been detected before the first half of the pigs were destroyed, but would certainly have been visible in the remaining

pigs by the time they were destroyed. Such changes were seen in pigs treated with the closely related agent ifosfamide in more recent studies (unpublished observations) by 3 weeks after dosing.

Serum alkaline phosphatase was only one of the enzymes mentioned by Hill (1975) which is known to be elevated by administration of cyclophosphamide. It was shown to be elevated in rats, dogs and monkeys by Lee et al., (1973) but although three out of the six treated pigs showed a slight rise soon after dosing, the levels of this enzyme stayed within the normal range.

Cyclophosphamide is known to be immunosuppressant, and to cause depletion of B-lymphocytes in the pig (Anderson et al., 1974) therefore it was expected that levels of serum globulins would fall markedly. This effect was not apparent at 3, 7 or 10 days after dosing, though had it been possible to measure globulins daily, a decrease might have been discovered. The increase in globulins noted may have been a "rebound" effect, though why there should have been a rise in all groups on day 21 is difficult to explain. The albumin levels on day 21 are very low, and any technical error in measuring the levels of albumin would result in falsely high calculated globulin levels.

Since urine samples were not taken, it was not possible to detect mild temporary cystitis or, micro-haematuria. However, only one pig (407) showed evidence of abnormality in the bladder at post-mortem and this was an animal which was given the lowest dose of cyclo-

phosphamide. The absence of bladder abnormalities in the pigs was disappointing, particularly as renal physiology in the pig is considered to be very similar to man (Nielsen, Maaske and Booth, 1966), but in the literature reports, summarised by Hill (1975), the percentage of human patients developing cystitis varied considerably, from 0.5% to 40%, and it has been recognised that the occurrence of cystitis in man does not directly correlate with dose level. One method of avoiding bladder toxicity in human patients is to ensure a high fluid intake before and during therapy. The pigs enjoyed playing with water, due to boredom, and it was not possible to estimate how much water the experimental animals actually consumed. The effect of restricting water intake was not investigated.

The metabolic pathway of cyclophosphamide is thought to be qualitatively identical in man and animals (Hill, 1975). Nevertheless, Hill's summary of the dose levels which are toxic in different species suggests that there is much quantitative variation. In mice, the single LD_{50} given intraperitoneally is 405mg/kg. In guinea-pigs, dosed intravenously, it is 400mg/kg, but in rats, it is only 150 to 180mg/kg and in rabbits 130mg/kg. In dogs, the LD_{50} is 40mg/kg, but in monkeys 240mg/kg is described as a lethal dose. The maximum tolerated dose in man is 27 to 41 mg/kg (1 to 1.5 g/m^2), therefore it appears that the dog resembles man more closely than either the monkey or the pig in this respect. It is interesting that the dog also appears to be the species most prone to cyclophosphamide - induced cystitis.

The effects on the thymus shown at post-mortem were less pronounced than those noted with prednisolone (See Chapter I) but were similar, with an "overshoot" after treatment was stopped, in the medium and high dose animals. This finding, and the temporary depression of skin reactions to DNFB in these groups might suggest that T-cell as well as B-cell activity may be affected by cyclophosphamide. However, as Milton et al., (1976) pointed out, the complex interactions between T-cells and B-cells make it difficult to assess what is a direct effect on one cell population.

The very slight effect of the drug on relative lymph node weights in the treated pigs probably did not reflect the effects on the lymphocytes in these nodes, because the framework of reticulum cells would be unaffected.

Despite the lack of toxicity observed in this experiment, the relatively low dose level of 20mg/kg was chosen for therapy of the lymphosarcoma cases, because of their poor clinical and haematological condition.

CYCLOPHOSPHAMIDE THERAPYMaterials and MethodsAnimals and Pre-treatment Procedures

For the therapy trial, four pigs were used. All were cases of lymphosarcoma which were diagnosed before 8 weeks of age. Three were females from the same litter, and the remaining pig was a younger entire male by the same sire. The dams of both litters were closely related. By the age of 10 weeks, they had all been transferred from the breeding unit to the experimental animal house. They were then kept in isolation in individual pens and allowed to become accustomed to their surroundings for several weeks. Management was as described in "General Methods". During this time, the pigs were sensitised with DNFB.

After overnight starvation, they were anaesthetised and staging laparotomy was carried out as described on pages 81 to 83 in "Methods". The diagnosis of lymphosarcoma was confirmed, and all four animals had widespread abdominal tumour involvement, with gross enlargement of mesenteric and gastric lymph nodes, spleen and splenic lymph nodes. Invasion of the liver was suspected, particularly in pig 420, because of the large size of the organ and its pale mottled appearance. A small piece of mesenteric lymph node was removed for histopathology and placed in 10% formalin.

Weekly weighing and blood sampling for haematology commenced in the three older animals from the date of surgery 3 weeks before the start of treatment. The operation was carried out only 10 days before the start of treatment in the younger male, because of dyspnoea under anaesthesia as a result of upper respiratory tract infection, but weekly blood-sampling was carried out on the same days as in the other three animals. Clotted blood samples for estimation of serum alkaline phosphatase, total protein and albumin, were taken on days - 14, - 7 and 0 and blood urea was measured from the haematology samples. Weekly detailed clinical examinations and recording of rectal temperatures, abdominal and lymph node measurements commenced at the same time. Fitness, appetite, state of urine and faeces and any abnormal behaviour were noted daily. If a pig was seen to be dull or off food, the rectal temperature was recorded and a detailed examination was carried out, so that supportive therapy could be administered promptly if required.

Pretreatment skin tests were carried out 7 days before the first dose of cyclophosphamide, except in pig 420.

Treatment

As in the trial with normal pigs, the preparation used was "Endoxana", and the drug was prepared for injection as previously described. The dose level used was 20 mg/kg in all four pigs.

The pigs were tranquillised and dosed as previously described in the normal pig trial, on day 0, day 17 and day 37.

During the treatment period, weekly weighing and clinical examinations were continued. The pigs were blood sampled more frequently than during the pretreatment period. Complete blood counts, urea, serum alkaline phosphatase, total protein and albumin were measured on days 7, 14, 21, 27, 34 and 41, and weekly thereafter in pig 362, which remained alive. In addition, haematology samples for WBC counts, differential counts and PCV were taken on days 1, 2, 3, 4, 5 and 6 after the first dose, on day 22 after the second dose and on days 42 and 43 after the third dose. Additional alkaline phosphatase estimations were carried out on serum samples on days 1 and 2.

DNFB skin tests were repeated on the day of the first dose, as it was considered that effects on the lymphoid system might develop rapidly, and again 19 days after the second dose.

When it was considered that the maximum effect of the third dose of cyclophosphamide would be easily demonstrated, two of the four pigs, 357 and 420, were

destroyed, by pentobarbitone overdose, and autopsied. Another laparotomy was carried out on pigs 359 and 362, so that effects of the drug on the tumour could be assessed in all four pigs. It was intended that these two animals should be left without treatment until they relapsed. However pig 359 did not recover from the laparotomy, and so only one animal was left alive.

It was finally destroyed nearly 8 weeks after the last dose of cyclophosphamide. Weekly weighing, clinical examinations and blood sampling were continued during this time and another skin test was carried out 2 days before it was killed.

RESULTS

Clinical Examinations (For clinical records, see Appendix III)

Pig No. 357 This female had shown signs of an attack of diarrhoea on the morning when staging laparotomy was planned. The operation was uneventful, but during the following day it vomited, and developed severe diarrhoea with clots of blood and necrotic debris being passed. It was treated with intramuscular chloramphenicol and multivitamins with large doses of kaolin by mouth. It became dehydrated and refused to eat for a day, but gradually recovered, after being tempted to eat with milk puddings and piglet creep. After 7 days it was eating normally and medication was changed to neomycin by mouth for 5 days. It had become very thin, but began to regain lost condition slowly as its appetite improved, and during the treatment period it remained well apart from a mild catarrhal conjunctivitis, which was treated daily with topical application of chloramphenicol ointment ("Chloromycetin" - Parke Davis).

Three days before the end of the treatment period, it developed diarrhoea again, but this improved with administration of neomycin in food.

Rectal temperatures stayed within the normal range in this animal.

Pig No. 359 This female remained well, with a good appetite, throughout the pretreatment and treatment periods. Rectal temperatures were within the normal

range. Recovery from the first staging laparotomy was uneventful, but after the second operation, it did not recover its appetite as expected, and 5 days later it started vomiting. It became very depressed and dehydrated, though it drank a great deal. The rectal temperature was 39.8°C initially but dropped to 37.5°C. Normal abdominal sounds were audible posteriorly, but only tinkling sounds of gas and fluid were audible in the anterior of the abdomen. Intestinal obstruction was suspected and the pig was reanaesthetised with azaperone intramuscularly and metomidate/intravenously ("Hypnodil", Janssen) to the required effect. The stomach was grossly dilated almost filling the abdominal cavity and the site of obstruction could not be identified, though there were widespread peritoneal adhesions, and attempts were made to break down some of these. The animal recovered from the anaesthetic by the following morning but there was no improvement in its condition, and so it was destroyed on humane grounds in extremis.

Pig No. 362 - This female remained well with a good appetite throughout the pretreatment and treatment periods. Rectal temperatures were within the normal range. Like 359, it underwent two staging laparotomies, and recovery from both operations was uneventful. Three weeks after the second operation, the pig developed severe diarrhoea and its rectal temperature rose to 41.0°C. It was given intramuscular injections of oxytetracycline for 3 days, followed by neomycin in

food for 5 days. It improved rapidly and remained well until it was destroyed at the end of the experiment. By this time, however, it was beginning to lose condition and had developed the typical "pot-bellied" appearance of an advanced lymphosarcoma case. See Plates 18 to 23.

Pig No. 420 - This male was already in poor condition as a result of the tumour at the commencement of the experiment. Staging laparotomy was delayed because of dyspnoea under anaesthesia due to upper respiratory tract infection, and was carried out only 10 days before the start of treatment. Three days after the operation, during which it ate little and passed no faeces, it started vomiting repeatedly. No bowel movements were heard on auscultation of the abdomen. Intestinal tract obstruction was suspected and the pig was reanaesthetised with azaperone intramuscularly and metomidate intravenously, to effect. When the abdomen was reopened at the same site, severe fibrinous adhesions were discovered. Part of the small intestine was adherent to the laparotomy incision, and it appeared that this was the site of obstruction. The adhesions were broken down and the wound resutured. Intramuscular ampicillin was administered for 3 days, and recovery was rapid with a return of normal appetite.

The pig remained well during therapy until 5 days after the second dose of cyclophosphamide when it developed a severe attack of diarrhoea. It did not lose its appetite and was treated orally with neomycin for 5

TABLE 43

Lymph Node measurements - mean products of length and breadths of inguinal and precrural nodes (mm^2).

WEEK	PIG NO. 357		PIG NO. 359		PIG NO. 362		PIG NO. 420	
	Inguinal	Precrural	Inguinal	Precrural	Inguinal	Precrural	Inguinal	Precrural
-2	Not Recorded		968	390	933	392	1059	348
-1	Not Recorded		944	416	1236	413	861	429
0	664	Not measurable	1509	525	1137	398	851	399
1st Dose								
1	674	Not measurable	946	413	787	351	1222	438
2	912	Not measurable	1011	432	1033	385	1472	544
3	780	Not measurable	1048	448	1229	450	1669	869
2nd Dose								
4	676	516	966	413	940	458	1467	636
5	830	572	1019	462	1074	594	1770	872
6	833	560	1326	479	1158	510	1938	912
3rd Dose				Not measurable				
7	Destroyed		947	measurable	1233	572	Destroyed	
8			Destroyed		1094	560		
9					1363	609		
10					1496	595		
11					1644	562		
12					2573	984		
13					2338	810		
14					Destroyed			

days, and kaolin. The faeces became more normal with this treatment, but from this time until it was destroyed at the end of treatment, it did not seem as lively as the others and its appetite was not as good as it had been earlier in the experiment. Its rectal temperature stayed within the normal range throughout the pre-treatment and treatment periods.

From Table 43, it can be seen that there was a shrinkage of the inguinal and precrural lymph nodes in the 1st week after the first dose in pigs 359 and 362, after the second dose in all four pigs, and after the third dose in pig 359 but not 362. The greatest response was after the first dose in pig 359, with a decrease in inguinal lymph node size of 37%. By the 2nd week after each dose however, the lymph nodes had increased in size again. The circumference of the abdomen in all four pigs increased steadily during the treatment period.

TABLE 44

Cyclophosphamide Therapy

Weight gains in kg. over 3-weekly intervals.

Pig No.	Pre-treatment	During treatment		After treatment
357	4.5*	9.0	1.0	Dead
359	8.5	6.0	4.0	Dead
362	9.0	6.5	4.0	8.0
420	3.5	4.5	5.0	Dead
* - ill during pre-treatment period.				

Weight gains

Effects of the treatment on weight gain were difficult to assess because of complicating factors (See Table 44). In two of the four pigs, 359 and 362, it appears that weight gain was definitely depressed during the treatment period. Pig 357 was ill during the pre-treatment period and put on weight rapidly after recovery despite the treatment. However its weight gain was depressed again after the second dose. The weight gain of pig 420 was apparently improved by the treatment, but like 357 its pre-treatment condition was affected by illness, due to post-operative complications.

DNFB Skin Test Results (See Table 45).

In two pigs, 357 and 362, there was an improvement in response after the first dose and a greater improvement by 19 days after the second dose. In 362, which was left alive 7 weeks after the last dose, this improvement was maintained, despite clinical and haematological evidence of relapse.

It was unfortunate that a pretreatment test result was not obtained from 420, as its first post-treatment response was good (score 3) and its second post-treatment response was even better (score 4).

Pig 359 had a negative response 2 days after the first treatment, and this was even poorer than the slight response recorded before treatment in this animal.

TABLE 45

Cyclophosphamide Therapy

DNFB Skin-Tests-Results

Pig No.	At Sensi- tisation	Pretreat- ment	Post-treat- ment 1*	Post-treat- ment 2+	7 weeks after last treatment
357	1	1	2	3	Dead
420	0	Not tested	3	4	Dead
359	1	1	0	2	Dead
362	1	1	2	3	3

* - 1 to 2 days after first dose

+ - 20 to 21 days after second dose

However, after two doses, an improvement to a score of 2 was obtained.

It appears, therefore, that although the results of post-treatment test 1 are not clear cut, by the time of post-treatment test 2 all pigs showed a definite improvement in skin hypersensitivity reaction.

Haematology

(See Appendix III Tables 35 to 42).

As previously noted in the experiment with normal pigs, WBC counts were depressed in all the pigs after each dose of cyclophosphamide. Figures 21 and 22 show mean \pm S.D. of mononuclear and neutrophil counts before and during treatment. From these it is clear that the nadir occurred less than 5 days after each dose, the lowest mononuclear counts occurring slightly earlier than the lowest neutrophil counts. These results were similar to those obtained in the normal pigs. Neutrophil counts were again depressed much more than the mononuclear cell counts, and at the nadir it was difficult to find any neutrophils at all when performing a differential count. For around 1 week after each dose in all pigs, morphologically abnormal lymphocytes were absent from blood smears. However, as the mononuclear cell counts increased again, tumour cells tended to reappear, in small numbers, particularly in those from pig 420.

Mean \pm 1 S.D. of RBC counts and Hb before and during treatment are shown in Figure 23 and PCV in Figure 24. From these, it can be seen that the pigs became more anaemic during the 3 weeks of the pretreatment period. Mean PCV increased slightly during the seven days after dosing, though RBC counts in three of the four pigs were lower on day 7 than on day 0. On the 14th day after

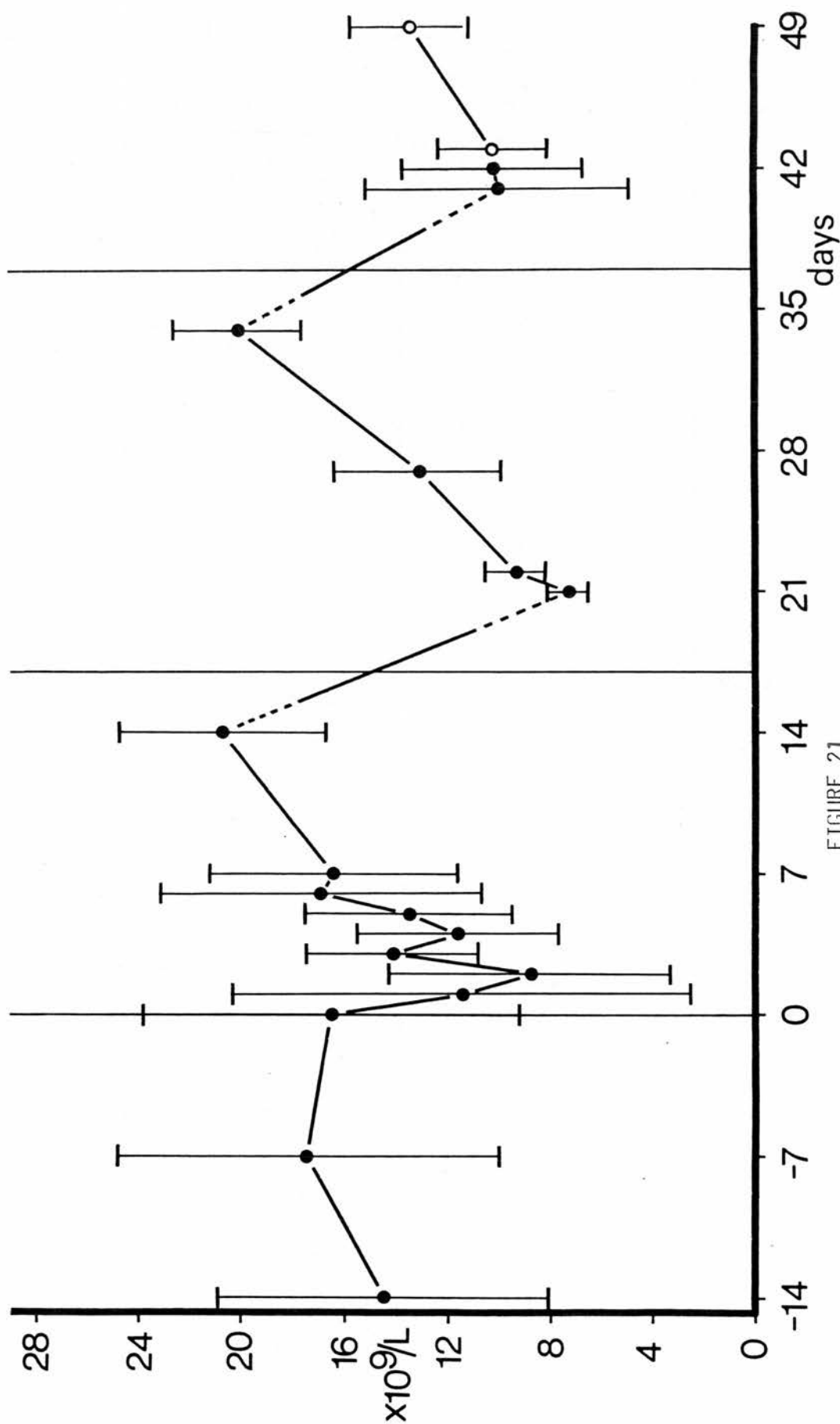


FIGURE 21

Absolute mononuclear cell counts (means \pm 1 S.D.) of four lymphosarcoma cases treated with 20mg/kg cyclophosphamide. Open symbols - two pigs. Vertical lines indicate times of dosing.

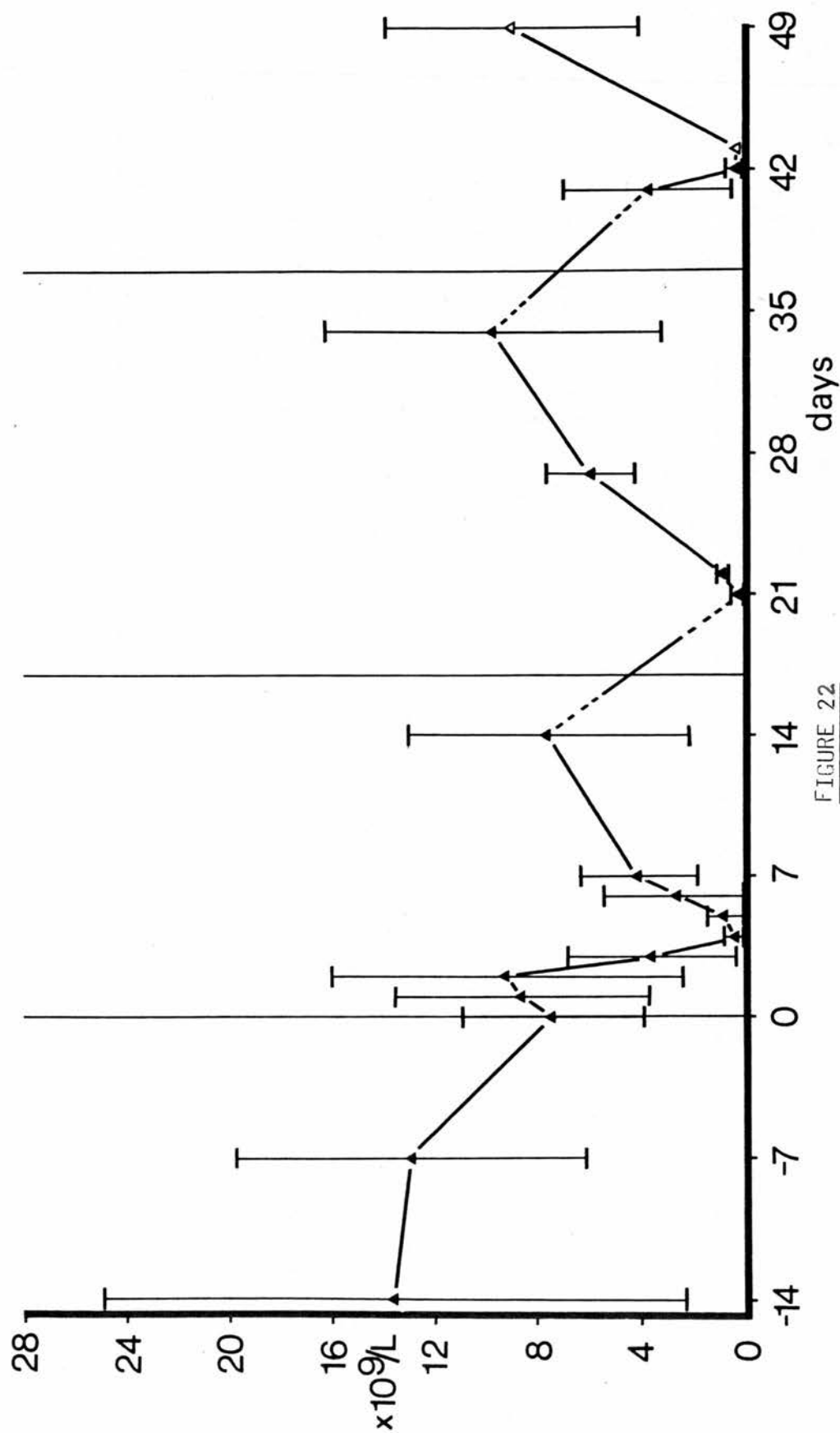


FIGURE 22

Absolute neutrophil counts (means \pm 1 S.D.) of four lymphosarcoma cases treated with 20mg/kg cyclophosphamide. Open symbols - two pigs. Vertical lines indicate times of dosing.

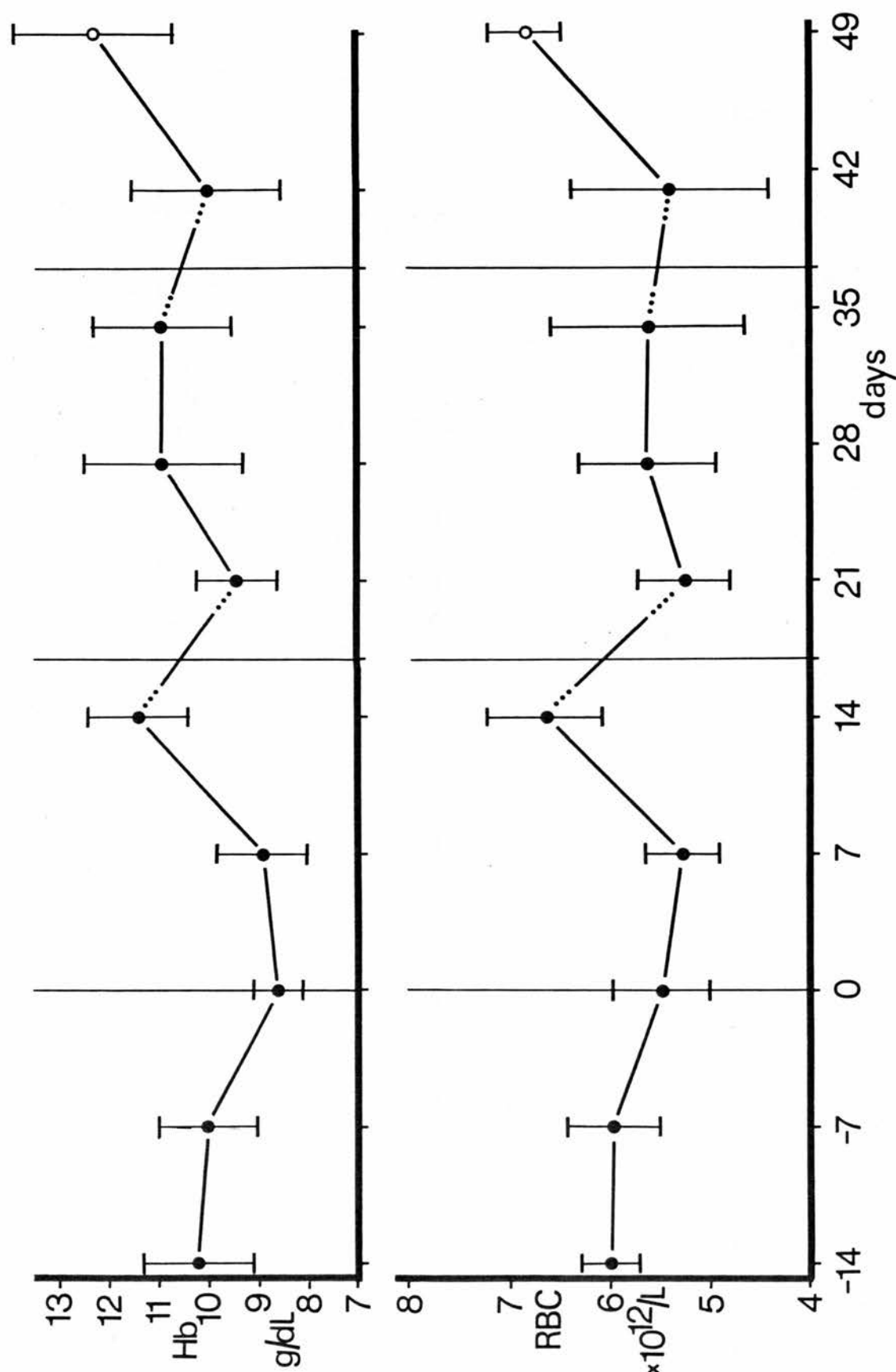


FIGURE 23

Hb and RBC counts (means ± 1 S.D.) of four lymphosarcoma cases treated with 20mg/kg cyclophosphamide. Open symbols - two pigs. Vertical lines indicate times of dosing.

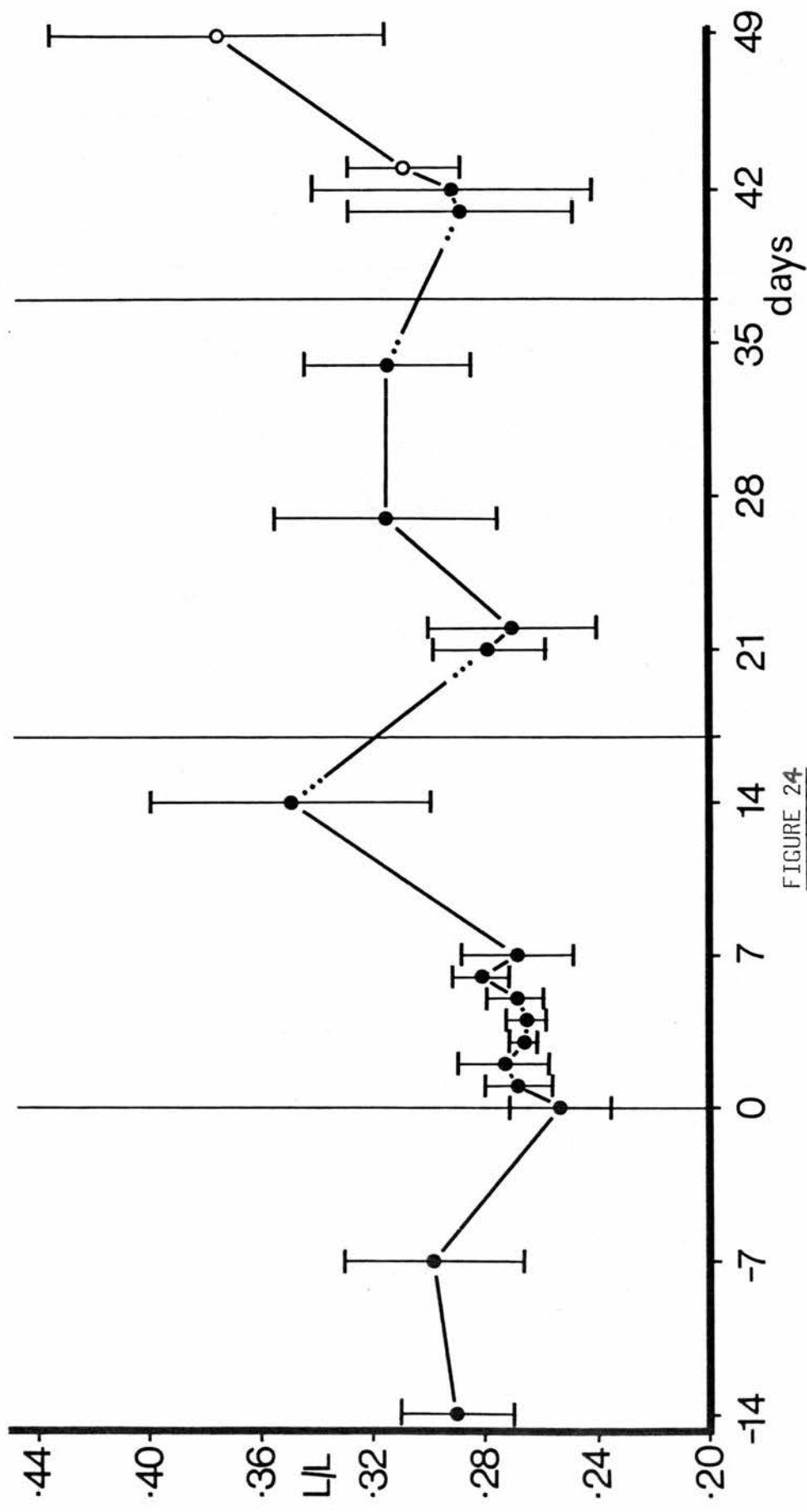


FIGURE 24

PCV (means \pm 1 S.D.) of four lymphosarcoma cases treated with 20mg/kg cyclophosphamide. Open symbols - two pigs. Vertical lines indicate times of dosing.

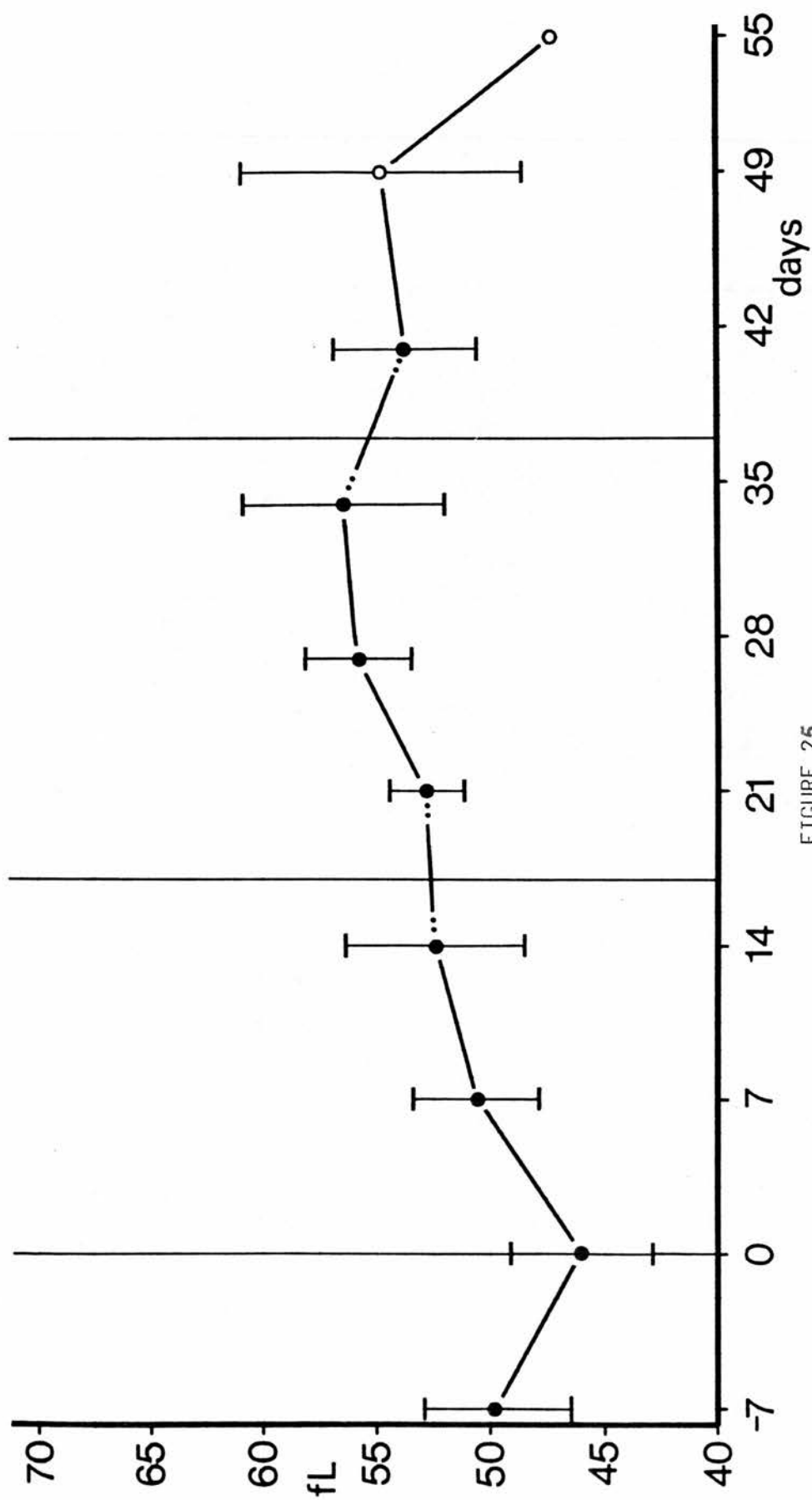


FIGURE 25

MCV (means \pm 1 S.D.) of four lymphosarcoma cases treated with 20mg/kg cyclophosphamide. Open symbols - two pigs. Vertical lines indicate times of dosing.

dosing, in all four pigs, RBC counts, PCV and Hb were much increased. After the second and third doses, these parameters were depressed, increasing again during the following 2 weeks, except in pig 420 which became progressively more anaemic despite therapy until it was destroyed. The high red cell values recorded in pig 359 on day 49 were undoubtedly due to dehydration, as a result of repeated vomiting.

When the values from 362 are examined, the pig which was left alive after treatment was stopped (See Table 42 Appendix III) it can be seen that 19 days after the third dose, its red cell picture was not very different from that of a normal pig. After this however it deteriorated, and when it was destroyed, nearly 8 weeks after the last dose it was once again moderately anaemic.

Means \pm 1 S.D. of MCV are shown in Figure 25. There was a progressive increase in this derived red cell index during treatment. There was no decrease in MCHC or MCH.

There was a wide variation in thrombocyte counts (See Appendix III) between pigs, and also in the same animals from week to week. Counts during the treatment period were within the normal range. The lowest count obtained ($91 \times 10^9/l$) was in pig 362, 2 days before it was destroyed, in relapse, after therapy was stopped.

Serum Proteins - Albumin and Total Globulins (See Appendix III).

Total serum globulin values were obtained, as before,

by subtraction of the measured values of serum albumin from the total protein values. One serum sample, from pig 420, was missing but it can be seen from the results of the other three pigs that by 7 days after the start of treatment, there was a fall in serum globulins and a rise in serum albumin. Considering only the three pigs for which results are complete, the albumin/globulin ratio altered from a mean of 0.54 ± 0.02 on day 0 to 1.61 ± 0.22 on day 7. By day 14, globulins had increased again, but the rise in albumin levels was maintained until day 14 and then fell slightly on day 21.

Serum Alkaline Phosphatase (See Appendix III)

On days 1 and 2, after the first dose of cyclophosphamide, temporary increases in SAP levels were noted in three out of the four pigs, but they were still within the normal range, with the exception of pig 359, which had a value of 201 iu/litre. By day 7 the levels had fallen considerably. None of the pigs showed increased levels after the second dose and only pig 362 had a very slight increase in SAP after the third dose. However, the pigs were not sampled until 4 days after the second and third doses, and increased levels may have been missed. In general, the SAP estimations tended to decrease progressively with age, as noted previously.

Blood Urea (See Appendix III, Tables 39 to 42).

Blood urea measurements were within the normal range throughout the experiment, except for those obtained from pig 357 on day -14, and 359 on day 48, which were slightly increased. On those particular days, the animals concerned were suffering from severe intestinal dysfunction (See Clinical Observations, page 295).

Post Mortem Findings:Pig No. 357

This was a rather small but anatomically normal, well developed, well nourished carcass.

The thymus was of moderate size with soft pale fawn lobules.

There was a small amount of serous fluid in the pericardial sac.

The heart muscle was flabby but otherwise normal.

The lungs, liver, adrenals, and bladder showed no abnormality, and the ovaries and uterus were small and inactive.

The spleen was of moderate size, smooth and firm. The cut surface showed engorgement with blood and no visible white pulp.

There were marked fibrous adhesions between the spleen and the right flank.

The kidney cortex was slightly pale, but the organ was otherwise normal.

The stomach showed no abnormality, but there was kinking of the small intestine by fibrous adhesions, though there was no stenosis and dilatation.

The caecum and colon were normal.

The bone marrow of the femur was pink and white throughout its length.

Pig No. 359

This was a well-developed but thin and dehydrated carcass with a very hairy coat.

The head and neck showed no abnormality but the brain was congested.

There were two surgical wounds in the abdomen one on the right lateral side, the other in the mid -line.

There was evidence of stomach contents staining the snout and mouth.

There was no fluid in the chest or pericardial sac.

The lungs showed patchy partial collapse and congestion.

The heart was normal apart from the muscle being flabby.

The thymus was not found.

There was a diffuse fibrous peritonitis, with a more recent fibrinous peritonitis, involving the lateral right abdominal wall where the fibrosis predominated and the mid-line abdominal wall where the fibrin predominated.

The liver showed diffuse fibrinous adhesions between the liver and the lesser curvature of the stomach, and between the visceral surface of the liver and the entire spleen. There was also a dense fibrous adhesion between the distal segment of the spleen and the liver.

In this area the interlobular septae were thickened but elsewhere the liver was normal on cut surface.

The spleen was enlarged. The cut surface was red and cellular with indistinct 3 to 4mm white pulp nodules. There was no folding of the borders, and no nodules were noted along the hilus.

The kidney capsules and dorsal wall of the bladder were involved in the peritonitis, but otherwise these organs showed no abnormality.

There were many small Graafian follicles on the ovaries but the uterus was small and inactive.

The stomach was very distended with watery green/brown ingesta. The serosa showed a mild degree of fibrinous peritonitis and the mucosa was slightly flushed.

At the site of the pancreas there were adhesions. On cut surface, some small brown lobules, and other areas of haemorrhage, fibrin and yellow necrosis were found, but no normal pancreatic tissue.

The small intestine was distended with brown/green ingesta of watery consistency as far as the middle. After this point, the intestines were collapsed and empty as far as the lower ileum. The serosal surface was involved in an acute fibrinous peritonitis causing adhesions in the proximal coils. Fibrinous tags and adhesions were also present in the large intestine. Peyer's patches were slightly thickened.

The large intestine was distended with digested food. There were adhesions between the caecum and the right

abdominal wall.

All carcase lymph nodes showed slight bilateral symmetrical enlargement and on cut surface 50 to 70 percent was formed by white cortex, the remainder by light fawn medulla. The mesenteric, gastric and bronchial nodes were much enlarged, and bulging and cellular on cut surface.

The bone marrow of the femur was red/pink with cellular and fatty mottling throughout the whole of its length.

Pig No. 420

This was a small moderately developed, moderately nourished carcase. The blood was very thin and watery.

The thymus was of moderate size with pale soft lobules.

Heart, lungs, liver, adrenals, kidneys and bladder showed no abnormality.

There were adhesions between the ventral end of the spleen, which was folded over on itself, and the abdominal wall. These could be broken down but were fibrous. The spleen was of normal shape and the cut surface showed red cellular tissue with blood but no visible white pulp.

The only abnormality in the stomach was in the cardiac region which showed three irregular areas of black, slightly raised nodularity up to 3cm diameter with several 5mm yellow raised necrotic plaques.

The small intestine showed many adhesions to the right flank, which were easily broken down, but were of fibrous tissue. There was also firm white fibrous thickening of the mesentery attachment which distorted the coils, making it difficult to unravel the intestine.

The caecum and colon were normal.

The bone marrow of the femur was moderately red and cellular.

Pig No. 362

The head and neck including the brain showed no abnormality, though the tonsils were slightly thicker and more nodular than usual.

The thymus was of moderate size with pale fawn lobules.

There were extensive fibrous adhesions in the mid-line ventral abdomen between the abdominal wall and the spleen and some of the loops of the small intestine.

Lungs, heart, liver, adrenals and bladder showed no abnormality.

The kidney capsules stripped easily leaving a few 1-2mm white nodules visible on the surface, which extended a little way into the cut surface.

The ovaries showed small corpora lutea and many smaller Graafian follicles. The uterus was small and inactive.

The stomach was full of food. The oesophageal region was normal. The mucosa of the fundus was slightly congested but normal except for a 1cm stellate depressed ulcer.

The bile-stained mucosa of the duodenum was slightly thickened and corrugated by up to 3mm of white cellular tissue.

The small intestine showed no abnormality but the Peyer's patches of the ileum were moderately prominent.

The caecum and colon were normal.

The spleen was moderately large and engorged. The edges were not curled and there were no nodules along the hilar region. On cut surface there was a faint nodularity but no obvious white pulp.

All lymph nodes were enlarged and on cut surface showed a flat white field with some nodularity but no obvious medulla. There was no evidence of haemorrhage.

The bone marrow of the femur was red/white mottled and sank in formalin.

Lymph Node and Organ Weights

See Appendix III for absolute weights in grams. Table 46 shows the relative weights of lymph nodes and organs, compared with a range of weights from three normal pigs killed at similar ages and 14 untreated cases (mean and range).

From the results of the two pigs killed at the end of treatment, and the one that died shortly after this, it can be seen that cyclophosphamide therapy did reduce

TABLE 46

RELATIVE WEIGHTS OF LYMPH NODES AND ORGANS (cg/kg BODY WEIGHT) OF INDIVIDUAL LYMPH OSARCOMA PIGS TREATED WITH CYCLOPHOSPHAMIDE COMPARED WITH THREE NORMAL PIGS AND 14 UNTREATED CASES OF SIMILAR AGE

PIG NO	NORMAL PIGS		CYCLOPHOSPHAMIDE TREATED LYMPHOSARCOMA CASES				UNTREATED CASES	
	RANGE OF THREE CONTROLS	AGE AT DEATH (DAYS)	KILLED AT END OF TREATMENT	DIED	KILLED END EXP.	MEAN (RANGE) OF 14 CASES		
			357	420	359	362		
AGE AT DEATH (DAYS)	157 to 170	169	130	177	219	182 (156 to 199)		
LYMPH NODES :								
Mesenteric and Colonic	305 - 408	1158	2362	1172	6539	3620 (974 - 9435)		
Gastric and Splenic	28 - 42	423	671	435	2308	1329 (579 - 2605)		
Bronchial Group	6 - 14	49	109	50	299	91 (42 - 189)		
Total Splanchnic	361 - 449	1630	3142	1657	9146	5040 (1595 - 12229)		
Head Group	24 - 32	93	122	78	235	160 (76 - 268)		
Iliac Group	7 - 8	11	41	10	24	32 (8 - 53)		
Prescapular	3 - 7	6	6	2	5	11 (0.4 - 22)		
Precrural	2 - 5	7	21	5	21	19 (5 - 53)		
Inguinal	11 - 14	14	46	10	39	39 (8 - 82)		
Popliteal	1	2	10	1	10	8 (1 - 17)		
Total								
Superficial	49 - 66	133	246	106	334	266 (98.4 - 495)		
Thymus	114 - 192	64	92	-	76	25 (0 - 149)		
Spleen	122 - 432	614	1298	630	2524	1500 (616 - 2702)		
Liver	2076 - 3306	3632	5106	3798	6088	6016 (3968 - 10438)		
Kidney	153 - 179	263	285	323	264	290 (211 - 400)		
Adrenal	3 - 5	9	5	18	6	10 (6 - 22)		

the relative lymph node weights, particularly of those of the splanchnic group, compared with the mean value for untreated cases and the nodes of the pig which was allowed to relapse (362). However the weights were still within the range for untreated cases and much larger than the relative weights of the normal pig lymph nodes. The haematology results suggested that pig 420, the only male, did not respond to the therapy nearly as well as the females, and the relative lymph node weights from 420 also showed a poor response compared with the results from 357 and 359.

Apart from 359 which had been ill for several days before its death, the relative thymus weights of the treated pigs were greater than the mean value for untreated cases, though not as great as in the normal pigs. The thymus was still large in the relapsed pig 362.

Splenic and liver relative weights were greatly reduced in 357 and 359 but again there was a poor response in pig 420. The spleen and liver of 362 were massive, a further indication of rapid tumour regrowth.

Kidney weights appeared to be unaffected by the treatment, and those of 362 were no heavier than the rest despite gross evidence of tumour involvement.

DISCUSSION

Cyclophosphamide has been used to treat tumours in animals, particularly lymphosarcoma in dogs (See Pages 50 in Introduction). McClelland (1963) noted severe haemorrhagic cystitis in a treated dog but apparently did not recognise that this was a side-effect of the drug. More recently, the problem of cyclophosphamide cystitis in the dog and cat was discussed by Crow, Theilen, Madewell, Weller and Henness (1977).

As with the normal pigs, therapy with cyclophosphamide at a dose level of 20mg/kg did not result in any severe clinical toxicity, even in these advanced cases which were in poor condition at the beginning of the experiment. The tumour was undoubtedly affected by the drug but the response, indicated by the shrinkage of the superficial lymph nodes, was disappointing and temporary. In retrospect, the results might well have been improved by using a higher dose level, which would probably have been well tolerated.

As already discovered in the prednisolone therapy experiment, there was an improvement in the DNFB skin test response during treatment. Pig 420 had a score of 4 when tested for the second time, which would be regarded as a good response in a normal pig. It is interesting to note that this animal also had the largest thymus at post-mortem, though the organ was not as large as that of a normal pig. (See Table 46)

Many problems were caused by the after effects of laparotomy. Although excessive handling of the gut was avoided as much as possible, and every precaution was taken to keep the operations sterile, peritoneal adhesions occurred rapidly, particularly between the coils of the small intestine. These were severe enough in two of the pigs to cause complete intestinal obstruction. As stated in the Methods section, the laparotomies were undertaken to confirm the diagnosis and the stage of the disease, to mimic the procedures used on human patients, and to illustrate a similar response in those pigs which were not killed at the end of treatment, but in fact they yielded comparatively little information compared with the problems that they caused. Weight gains in particular were difficult to assess.

The haematology results were similar to those obtained in the normal pigs, i.e. after each dose, there was a depression of WBC, mononuclear and neutrophil counts. Tumour cells almost disappeared from the blood smears at this time, and the mononuclear cells which remained resembled small lymphocytes with a few typical monocytes. However, their reappearance before the next dose was administered indicated that the drug was only suppressing the tumour temporarily. It was surprising that there were no problems with opportunist infections during the first week after dosing when the neutrophil counts were very low indeed. Undoubtedly, the minimal disease status of the piggery helped, and if

the pigs had been exposed to ordinary farm conditions, they may well have succumbed to virulent infections.

As in the normal pigs, RBC, PCV and Hb were temporarily depressed after dosing, but by the 14th day a marked improvement had occurred in three out of the four animals, probably due to recovery of normal red cell precursors following destruction of infiltrating tumour tissue in the bone marrow.

CHAPTER IVCYTARABINEINTRODUCTION

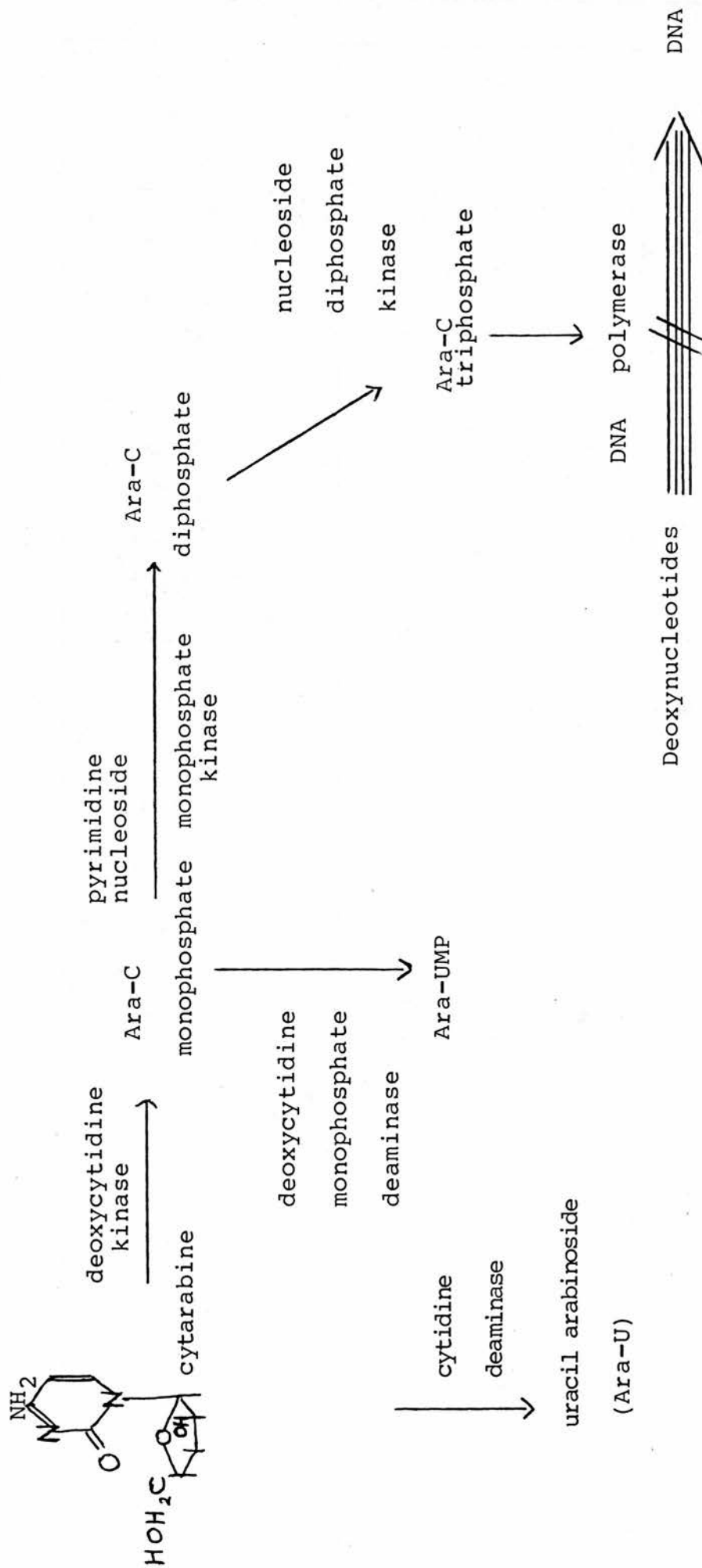
Cytarabine (also known as cytosine arabinoside, arabinosyl cytosine, 1- β -D-arabinofuranosylcytosine, Ara-C) is an example of the group of drugs known as antimetabolites. Cytarabine is a pyrimidine nucleoside analogue. Its structure is similar to that of cytidine and deoxycytidine except that the sugar moiety is arabinose rather than ribose or deoxyribose. Its antitumour activity is due to competitive inhibition by its active form, the triphosphate, of the enzyme DNA polymerase, preventing it from completing the final step in the assembly of DNA. The drug is converted within the cell by a multistep phosphorylation pathway (See Figure 25) involving three enzymes, the first of which is called deoxycytidine kinase. It is also subject to deactivation, however, by the enzyme cytidine deaminase, to uracil arabinoside.

Cytarabine is therefore a cell-cycle specific agent because it acts only in the S-phase of dividing cells. In addition, it blocks the progression of cells in G₁ into S-phase, resulting in a partial synchronisation of the cell population.

The literature about cytarabine was reviewed by Kremer (1975).

Cytarabine was synthesised in 1959, and Evans, et al., (1964) demonstrated that it was effective in

FIGURE 26



(Adapted from Chabner, et al., 1977).

inhibiting growth of several mouse tumours. Clinical trials began in 1964, and by 1969, several cancer chemotherapy groups had shown that cytarabine was an active agent in man, especially in the treatment of acute myeloblastic leukaemia.

The enzymatic reactions in the metabolic pathway of cytarabine may hold the key to the mechanisms of resistance to the drug, since they determine the amount of active product produced by the cells. In vitro studies by Kessel, Hall and Rosenthal (1969) showed a high ratio of deoxycytidine kinase to cytidine deaminase in lymphocytes, suggesting that cytarabine might be beneficial in lymphoproliferative disorders. It has been shown that there is great variability among human patients in the levels of kinase and deaminase in tumour cells. (Frei, Ho, Bodey and Freireich, 1973). Studies by Steuart, Burke and Owens (1971), and Ho (1973) yielded evidence that kinase and deaminase levels correlated well with clinical response in adult acute leukaemia. However a more recent study found no relationship between pretreatment enzyme levels and response (Smyth, Robins and Leese, 1976).

Although cytarabine is not regularly used for remission induction in ALL and lymphomas, it has been shown to be useful in patients who are resistant to standard therapy (Lay, Ekert and Colebatch, 1975). as one of a combination of agents used for maintenance therapy (Wollner, Burchenal, Leiberman, Exelby, D'Angio

and Murphy 1976), and in lymphomas which have a poor prognosis e.g. histiocytic (Levitt, Marsh, Deconti, Mitchell, Skeel, Farber and Bertino, 1972).

The pharmacokinetics of cytarabine are determined by cytidine deaminase, which is found in high concentration in liver, kidney and gastro-intestinal mucosa. It is ineffective when given orally. After rapid intravenous injection in man, it has a very short initial plasma half-life of 3 to 15 minutes, followed by a slower phase with a half-life of 2 to 2.5 hours (Ho and Frei, 1971, and Wan, Huffman, Azarnoff, Hoogstraten and Larsen, 1974). The drug is rapidly converted to uracil arabinoside and this metabolite becomes the predominant compound in plasma within minutes of drug administration. More than 90% of the dose, as uracil arabinoside, is excreted in urine within 24 hours.

Because of the S-phase specificity of cytarabine and its very short plasma half-life, it has to be administered frequently. To maintain effective blood levels, intravenous infusion is necessary (Ho and Frei, 1971, and Wan et al., 1974). It has been given intrathecally for meningeal leukaemia, as the drug persists in CSF for much longer periods.

The toxic action of cytarabine is directed at rapidly dividing tissues such as bone marrow and gastro-intestinal tract. Depression of white cells and thrombocytes may appear. Immunosuppression occurs,

though large doses are required to suppress cell-mediated immunity in laboratory animals (Griswald, Heppner and Calabresi, 1972).

The bone marrow effects are reversible with discontinuation of therapy. Long-term consequences of cytarabine administration are unknown but the drug produces striking cytogenetic abnormalities in bone-marrow cells, (Bell, Whang, Carbone, Brecher and Block, 1966). i.e. extensive fragmentation of chromatids, and therefore must be considered potentially carcinogenic.

Other adverse effects recorded in man are vomiting and diarrhoea, oral inflammation and ulceration, thrombophlebitis, hepatic dysfunction, fever and skin rashes (Upjohn Ltd., product information leaflet).

Using the information gained from the literature, trials were planned using normal and lymphosarcomatous pigs.

CYTARABINE IN NORMAL PIGSMATERIALS AND METHODS

Ten pigs were used, four females and six castrated males, five from each of two closely related litters born within 2 days of each other. The sire and both dams were of the lymphosarcoma strain but were non-carriers by breeding. The piglets were weaned at 8 weeks and housing, management and feeding were as described in the General Methods section.

As before, the pigs were allocated by lot to four groups, low-dose, medium-dose, high-dose and controls. However, different dose-levels were not used until the 3rd week of the experiment. For the first 2 weeks, all treated pigs were given the same dose of drug. The reason for this was to determine whether cumulative bone marrow toxicity would develop at the lower dose, before proceeding to higher doses. Continuation of the higher dose levels beyond one week was not possible because of limited drug availability. The control animals were given a similar volume of saline.

The pre-treatment procedures were as follows (see General Methods).

At 8 weeks of age, all the pigs were sensitised to DNFB, and they were skin-tested 10 days later. At 9 weeks of age, 4 weeks before treatment started, weekly weighing, and blood-sampling for WBC, RBC, PCV, Hb differential and thrombocyte counts, blood urea, and

serum protein estimations were commenced. SGOT and alkaline phosphatase were measured from the sample taken immediately before the start of treatment. The pigs were observed daily in the pen for appetite and general fitness, and state of urine and faeces.

A detailed clinical examination, including rectal temperature, condition of visible mucous membranes and auscultation of heart and lungs, was carried out weekly from 2 weeks before the start of treatment.

One week before treatment, the right inguinal lymph node was removed from each animal. Local anaesthetic solution, 2% lignocaine ("Xylocaine", Astra) was infiltrated subcutaneously around the lymph node. The skin was incised, and the node separated from subcutaneous fat by blunt dissection. Blood vessels were clamped with artery forceps and tied off with catgut (Ethicon Ltd.). The skin incision was closed with single interrupted sutures of 2.5 nylon, and an intramuscular injection of 2ml benzathine penicillin was administered. For the first 2 or 3 days after the node was removed, there was marked swelling caused by lymph accumulation, and leakage of fluid from the wound, which resulted in the wound becoming infected in six animals. This was treated with further penicillin injections in the most severely affected, removal of some of the stitches to allow drainage and dusting with

chlortetracycline powder ("Aureomycin", 2% Cyanamid).

The excised lymph nodes were wrapped in aluminium foil and frozen at -40°C for estimation of deoxycytidine kinase (DCK) at a later date.

Drug administration commenced when the pigs were 13 weeks old.

The preparation used was "Cytosar" (Upjohn Ltd., Crawley). Each vial contained 100mg freeze-dried cytarabine powder, which was dissolved immediately before use in 5ml water for injection. The powder dissolved easily, giving a solution of 20mg/ml.

Upjohn Ltd. recommend in their instruction leaflet a starting dose of 2mg/kg/day for continuous treatment and 3 to 5mg/kg/day for intermittent treatment in man, this to be continued for 10 days or until signs of toxicity appear. If no toxicity appears, the dose is increased.

The animals were first allocated to a treatment group and a control group, as follows:-

Pigs	298)	
)	
	300)	
)	
	306)	
)	
	307)	- 50 mg (2.5ml) Cytarabine/day
)	
	310)	
)	
	312)	

Pigs 304, 305, 308, 309 - 2.5 ml saline/day.

As all the treatment group pigs weighed between 20 and 25 kg at the start of dosing, 50 mg was chosen

as being a suitable and convenient dose for initial study.

The drug was administered by subcutaneous injection in the side of the neck, after swabbing the skin with methylated spirit. Half of the daily dose was administered in the morning and half in the late afternoon. This was continued for 5 days and not administered at the weekend, then given for a further 5 days.

Blood-sampling was carried out daily, before the morning injection, on weekdays only. WBC, differential, RBC and thrombocyte counts, PCV and Hb were recorded, and once weekly a larger sample was taken for serum total protein and albumin, SGOT and alkaline phosphatase measurements. Blood urea estimations were carried out weekly on the haematology samples. Weekly estimations and weighing were continued.

At the start of the 3rd week, the treated pigs were allocated to low-medium-and high-dose groups as follows:-

Pigs 307)
) Group A - 50mg/day as before
 312)

Pigs 300)
) Group B - 70mg/day
 310)

Pigs 298)
) Group C - 90mg/day
 306)

As before, the increased doses were administered subcutaneously, half in the morning and half in the late

afternoon. Daily blood-sampling for haematology was continued.

During the 1st and 3rd weeks of treatment, DNFB skin tests were carried out, and at the end of the 3rd week, the left inguinal lymph nodes were removed from two control animals and one animal from each of the treated groups. These pigs were given no further treatment and were left to recover from the effects of the drug. The remaining five pigs were destroyed by pentobarbitone overdose and autopsies were performed. The controls were killed on day 17 and the treated animals on day 18.

The surviving pigs were blood-sampled three times during the following week on days 21, 23 and 35, and twice during weeks 5, 6 and 7. At the end of the 7th week from the start of dosing i.e. after 4 weeks of recovery, these pigs were skin-tested once more, then destroyed and autopsied.

Enzyme Estimations

Estimation of DCK levels in lymphoid tissue from the normal pigs, and also from lymphosarcoma pigs, was carried out by Dr. A. Baxter at the Department of Clinical Oncology, University of Glasgow, 1 Horselethill Road, Glasgow.

The enzyme was assayed by the disc method of Durham and Ives (1969).

Briefly, the method was as follows. Homogenised tissue was incubated with a reaction mixture containing ^3H -deoxycytidine or ^3H -cytarabine, and after the reaction was stopped by heating, the mixture was centrifuged. The phosphorylated reaction products were quantitated by placing aliquots of the supernatant on to paper discs, washing, adding scintillation solvent and counting in a liquid scintillation counter. In further experiments, the enzyme was partially purified, by the method of Durham and Ives (1970), as far as fraction V. Kinetic studies were carried out and K_m values (dissociation constants) were determined for the enzyme in tumour tissue compared with normal tissue, i.e. this was a measure of the affinity of the enzymes for the substrates.

RESULTS

Clinical Examinations (For clinical records, see Appendix IV)

Throughout the experiment, all the pigs remained fit with excellent appetites. Rectal temperatures stayed within the normal range, below 40°C. Faeces and urine appeared normal. Pig 298 had bursitis on the posterior aspect of the right tarsus, which was inflamed throughout the experiment but this caused little inconvenience to the animal. The only problems were associated with poor healing of the wounds made to remove the inguinal lymph nodes, as already described and this occurred in two out of the four control animals, as well as in the treated group.

DNFB Skin Test Results

It can be seen from Table 48 that the reaction scores in the control animals were rather variable particularly in 304 and 309, which showed decreases in response during the treatment period. This may have been due to the pigs becoming wet in the pen immediately after the DNFB was applied. The two low-dose pigs also showed a decrease in reaction score during treatment, compared with their pretreatment scores, but the remaining treated pigs showed similar or increased responses. It appears therefore that cytarabine did not affect cell-mediated response to DNFB at these dose levels.

TABLE 47

CYTARABINE IN NORMAL PIGS - BODY WEIGHT GAINS

Pig No.	Dose Level	Wt. 3 wks. of experiment (kg)	Wt. at start of treatment (kg)	Wt. at end of treatment (kg)	Gain	Wt. 3 wks from end of treatment	Gain	Total Gain
298	High	19.5	25	24	-1	33	9	13.5
306	"	17	20.5	19	-1.5	Dead	-	-
300	Medium	17	20.5	20	-0.5	28.5	8.5	11.5
310	"	16.5	20	19.5	-0.5	Dead	-	-
307	Low	20.5	23	24	1	33	9	12.5
312	"	20	25	27	2	Dead	-	-
304	Cont-rol	18	24	28.5	4.5	39	10.5	21
305	"	15.5	19.5	25	5.5	Dead	-	-
308	"	18	21.5	22.5	1	29.5	7	11.5
309	"	13	15.5	18	2.5	Dead	-	-

TABLE 48

CYTARABINE IN NORMAL PIGS - DNFB SKIN-TEST RESULTS

Groups	Pig Number	At Sensitisation	Pre-treatment	During Treatment 1*	During Treatment 2*	Post-treatment
Treated	A 307	2	3	2	1	2
	312	2	3	1	2	Dead
	B 300	2	2	2	4	2
	310	2	2	4	3	Dead
C	298	2	2	3	3	2
	306	2	3	3	2	Dead
	304	2	4	3	2	3
	305	2	3	4	4	Dead
Control	308	2	4	4	2	3
	309	3	3	2	1	Dead

* 1 - all treated pigs given same dose level

2 - different dose levels used.

Body weight gains

From Table 47 it can be seen that during treatment the controls and low-dose pigs gained weight and the medium- and high-dose pigs lost weight. In three out of the four controls, body weight gain was greater than in either of the low-dose animals. This effect of cytarabine on weight was marked considering the short period of treatment but it was only temporary. Three weeks after the end of treatment the remaining animals' weight gains had improved dramatically.

Haematology

Cytarabine administration produced a progressive decrease in total WBC counts and in absolute neutrophil, lymphocyte and monocyte counts, which became apparent from the 4th day of treatment onwards. See Appendix IV for results from individual pigs. Figure 27 compares mean WBC counts of all the treated animals with mean counts of the saline-treated controls. Mean total WBC counts and mean absolute counts of neutrophils, lymphocytes and monocytes \pm 1 S.D. are shown in Table 49, from the controls and all treated pigs in weeks 1 and 2 and from low-, medium- and high-dose groups in week 3. Figure 27 does not show the mean counts of the separate groups in week 3, or standard deviations where they would have confused the picture. From Table 49, it can be seen that, although the mean counts of all the treated groups were lowest in week 3, the effect did not appear to be dose-related, being most marked in the medium-dose group.

After dosing was stopped, the counts remained low for a further week, in the surviving animals from the treated groups, and then began to recover rapidly. By the end of the experiment the counts of all types of WBC in the treated pigs were indistinguishable from those of the remaining controls.

Table 50 shows mean RBC counts PCV, Hb and MCV, MCHC and MCH values \pm 1 S.D. of the control pigs and all

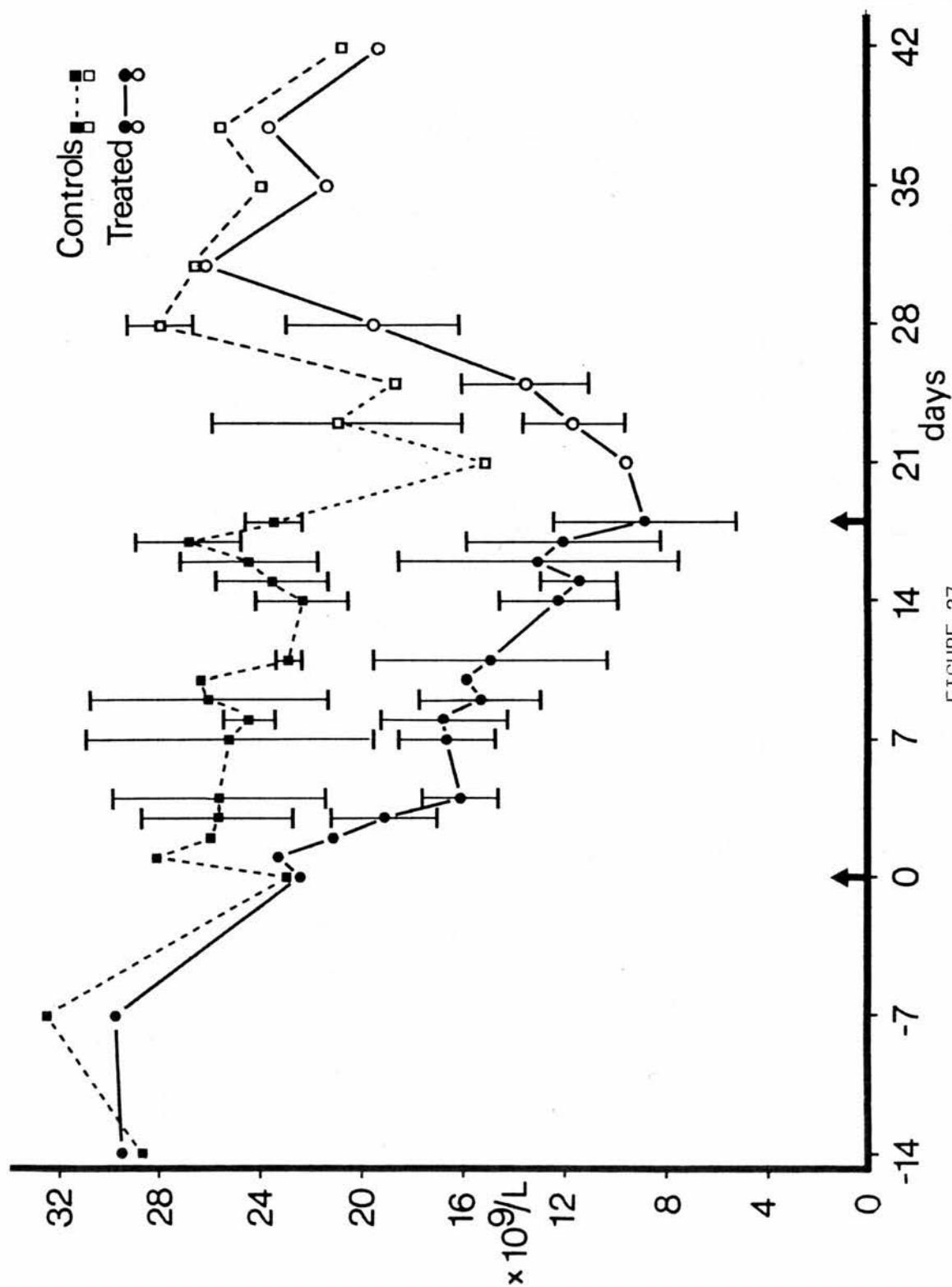


FIGURE 27

Mean WBC counts of six normal pigs treated with cytarabine compared with mean counts of four saline-treated controls. Arrows indicate beginning and end of treatment. Open symbols after half of the pigs were killed.

TABLE 49

EFFECTS OF CYTARABINE ON HAEMATOLOGY OF NORMAL PIGS

GROUP D - SALINE TREATED CONTROLS GROUP A - LOW DOSE GROUP B - MEDIUM DOSE GROUP C - HIGH DOSE

DAYS FROM START OF DOSING	WBC - MEANS + 1 S D $\times 10^9/L$				T NEUTROPHILS - MEANS + 1 S D				LYMPHOCYTES - MEANS + 1 S D				MONOCYTES - MEANS + 1 S D			
	GROUP D	GROUPS A, B&C	GROUP D	GROUPS A, B&C	GROUP D	GROUPS A, B&C	GROUP D	GROUPS A, B&C	GROUP D	GROUPS A, B&C	GROUP D	GROUPS A, B&C	GROUP D	GROUPS A, B&C	GROUP D	GROUPS A, B&C
- 28	28.5 \pm 5.7	29.7 \pm 3.3	12.3 \pm 5.2	15.6 \pm 1.7	14.0 \pm 3.0	11.2 \pm 2.5	11.9 \pm 0.8	11.2 \pm 2.5	11.2 \pm 2.5	11.2 \pm 2.5	11.2 \pm 2.5	11.2 \pm 2.5	1.9 \pm 0.8	2.1 \pm 0.6	1.9 \pm 0.8	2.1 \pm 0.6
- 21	26.7 \pm 4.1	27.1 \pm 4.6	14.1 \pm 6.4	11.9 \pm 2.6	11.2 \pm 1.9	13.0 \pm 3.0	13.0 \pm 3.0	13.0 \pm 3.0	13.0 \pm 3.0	13.0 \pm 3.0	13.0 \pm 3.0	13.0 \pm 3.0	1.1 \pm 0.8	1.7 \pm 0.6	1.1 \pm 0.8	1.7 \pm 0.6
- 14	28.7 \pm 2.6	29.5 \pm 5.2	11.8 \pm 2.1	7.9 \pm 6.9	15.5 \pm 2.2	13.9 \pm 4.1	13.9 \pm 4.1	13.9 \pm 4.1	13.9 \pm 4.1	13.9 \pm 4.1	13.9 \pm 4.1	13.9 \pm 4.1	1.1 \pm 0.4	2.2 \pm 1.5	1.1 \pm 0.4	2.2 \pm 1.5
- 7	32.5 \pm 7.4	29.7 \pm 1.6	20.1 \pm 8.8	15.0 \pm 3.3	11.8 \pm 2.6	12.1 \pm 1.8	12.1 \pm 1.8	12.1 \pm 1.8	12.1 \pm 1.8	12.1 \pm 1.8	12.1 \pm 1.8	12.1 \pm 1.8	1.8 \pm 1.0	2.0 \pm 0.8	1.8 \pm 1.0	2.0 \pm 0.8
0	22.9 \pm 4.5	22.4 \pm 2.4	8.8 \pm 3.1	7.5 \pm 1.3	12.6 \pm 1.7	12.9 \pm 2.6	12.9 \pm 2.6	12.9 \pm 2.6	12.9 \pm 2.6	12.9 \pm 2.6	12.9 \pm 2.6	12.9 \pm 2.6	1.2 \pm 0.4	0.8 \pm 0.3	1.2 \pm 0.4	0.8 \pm 0.3
Start of Dosing																
1	28.1 \pm 5.3	23.3 \pm 2.7	11.4 \pm 1.4	10.3 \pm 2.4	15.0 \pm 5.0	11.5 \pm 1.7	15.0 \pm 5.0	11.5 \pm 1.7	15.0 \pm 5.0	11.5 \pm 1.7	15.0 \pm 5.0	11.5 \pm 1.7	1.2 \pm 0.6	1.0 \pm 0.4	1.2 \pm 0.6	1.0 \pm 0.4
2	25.9 \pm 1.8	21.2 \pm 5.0	13.0 \pm 1.9	9.8 \pm 3.9	10.4 \pm 2.7	9.9 \pm 1.4	10.4 \pm 2.7	9.9 \pm 1.4	10.4 \pm 2.7	9.9 \pm 1.4	10.4 \pm 2.7	9.9 \pm 1.4	2.2 \pm 0.4	1.1 \pm 0.7	2.2 \pm 0.4	1.1 \pm 0.7
3	25.7 \pm 3.0	19.1 \pm 2.1	10.4 \pm 2.8	8.7 \pm 1.8	13.5 \pm 2.2	8.9 \pm 1.6	13.5 \pm 2.2	8.9 \pm 1.6	13.5 \pm 2.2	8.9 \pm 1.6	13.5 \pm 2.2	8.9 \pm 1.6	1.2 \pm 0.3	0.9 \pm 0.4	1.2 \pm 0.3	0.9 \pm 0.4
4	25.6 \pm 4.2	16.1 \pm 1.5	10.1 \pm 3.0	5.5 \pm 1.0	12.7 \pm 2.5	9.0 \pm 1.2	12.7 \pm 2.5	9.0 \pm 1.2	12.7 \pm 2.5	9.0 \pm 1.2	12.7 \pm 2.5	9.0 \pm 1.2	2.2 \pm 0.7	1.2 \pm 0.6	2.2 \pm 0.7	1.2 \pm 0.6
7	25.2 \pm 5.7	16.6 \pm 1.9	8.9 \pm 2.5	6.6 \pm 0.8	14.6 \pm 3.3	8.2 \pm 1.5	14.6 \pm 3.3	8.2 \pm 1.5	14.6 \pm 3.3	8.2 \pm 1.5	14.6 \pm 3.3	8.2 \pm 1.5	1.3 \pm 0.3	1.3 \pm 0.4	1.3 \pm 0.3	1.3 \pm 0.4
8	24.4 \pm 1.0	16.7 \pm 2.5	8.2 \pm 1.1	6.2 \pm 1.9	14.3 \pm 1.1	8.7 \pm 1.6	14.3 \pm 1.1	8.7 \pm 1.6	14.3 \pm 1.1	8.7 \pm 1.6	14.3 \pm 1.1	8.7 \pm 1.6	1.5 \pm 0.4	1.5 \pm 0.8	1.5 \pm 0.4	1.5 \pm 0.8
9	26.0 \pm 4.7	15.3 \pm 2.4	10.1 \pm 2.0	5.1 \pm 0.9	13.7 \pm 3.8	8.8 \pm 1.4	13.7 \pm 3.8	8.8 \pm 1.4	13.7 \pm 3.8	8.8 \pm 1.4	13.7 \pm 3.8	8.8 \pm 1.4	1.9 \pm 0.8	1.3 \pm 0.4	1.9 \pm 0.8	1.3 \pm 0.4
10	26.3 \pm 5.6	15.9 \pm 5.8	9.1 \pm 0.9	7.4 \pm 6.5	15.0 \pm 5.7	7.3 \pm 1.6	15.0 \pm 5.7	7.3 \pm 1.6	15.0 \pm 5.7	7.3 \pm 1.6	15.0 \pm 5.7	7.3 \pm 1.6	1.9 \pm 0.5	1.1 \pm 0.3	1.9 \pm 0.5	1.1 \pm 0.3
11	22.8 \pm 0.5	14.9 \pm 4.6	7.6 \pm 1.0	6.2 \pm 3.1	13.6 \pm 1.2	7.7 \pm 1.7	13.6 \pm 1.2	7.7 \pm 1.7	13.6 \pm 1.2	7.7 \pm 1.7	13.6 \pm 1.2	7.7 \pm 1.7	1.1 \pm 0.4	0.8 \pm 0.7	1.1 \pm 0.4	0.8 \pm 0.7
Dose Increased																
14	22.3 \pm 1.8	13.0 \pm 4.3	8.3 \pm 0.8	4.7 \pm 1.7	12.3 \pm 1.8	7.6 \pm 2.1	12.3 \pm 1.8	7.6 \pm 2.1	12.3 \pm 1.8	7.6 \pm 2.1	12.3 \pm 1.8	7.6 \pm 2.1	0.9 \pm 0.4	0.5 \pm 0.6	0.9 \pm 0.4	0.5 \pm 0.6
15	23.5 \pm 2.2	12.3 \pm 1.6	9.3 \pm 1.2	3.5 \pm 0.6	12.3 \pm 1.8	8.2 \pm 0.6	12.3 \pm 1.8	8.2 \pm 0.6	12.3 \pm 1.8	8.2 \pm 0.6	12.3 \pm 1.8	8.2 \pm 0.6	1.2 \pm 0.4	0.4 \pm 0.1	1.2 \pm 0.4	0.4 \pm 0.1
16	24.4 \pm 2.7	16.4 \pm 9.5	7.9 \pm 1.2	7.7 \pm 6.4	13.6 \pm 1.9	8.2 \pm 3.1	13.6 \pm 1.9	8.2 \pm 3.1	13.6 \pm 1.9	8.2 \pm 3.1	13.6 \pm 1.9	8.2 \pm 3.1	1.5 \pm 0.4	0.3 \pm 0.1	1.5 \pm 0.4	0.3 \pm 0.1
17	26.8 \pm 2.1	14.4 \pm 6.3	9.0 \pm 1.2	5.7 \pm 3.7	15.7 \pm 1.9	7.8 \pm 2.3	15.7 \pm 1.9	7.8 \pm 2.3	15.7 \pm 1.9	7.8 \pm 2.3	15.7 \pm 1.9	7.8 \pm 2.3	1.9 \pm 0.6	0.7 \pm 0.4	1.9 \pm 0.6	0.7 \pm 0.4
18	23.4 \pm 1.1	11.0 \pm 6.2	8.4 \pm 0.3	4.6 \pm 3.9	13.3 \pm 0.3	5.7 \pm 1.7	13.3 \pm 0.3	5.7 \pm 1.7	13.3 \pm 0.3	5.7 \pm 1.7	13.3 \pm 0.3	5.7 \pm 1.7	1.1 \pm 0.6	0.4 \pm 0.1	1.1 \pm 0.6	0.4 \pm 0.1
End of Dosing																
21	15.1 \pm 5.6	8.3	4.8 \pm 2.6	0.8	8.4 \pm 2.1	6.7	8.4 \pm 2.1	6.7	8.4 \pm 2.1	6.7	8.4 \pm 2.1	6.7	1.3 \pm 0.3	0.3	1.3 \pm 0.3	0.3
23	20.9 \pm 4.9	13.3	6.3 \pm 0.5	2.1	13.2 \pm 5.6	9.2	13.2 \pm 5.6	9.2	13.2 \pm 5.6	9.2	13.2 \pm 5.6	9.2	1.1 \pm 0.6	1.3	1.1 \pm 0.6	1.3
25	18.6 \pm 0.1	16.0	7.4 \pm 0.1	5.1	9.2 \pm 0.1	9.4	9.2 \pm 0.1	9.4	9.2 \pm 0.1	9.4	9.2 \pm 0.1	9.4	1.6 \pm 0.1	1.3	1.6 \pm 0.1	1.3
28	27.9 \pm 1.3	22.3	12.7 \pm 4.9	11.6	14.2 \pm 2.5	9.6	14.2 \pm 2.5	9.6	14.2 \pm 2.5	9.6	14.2 \pm 2.5	9.6	0.8 \pm 0.8	1.1	0.8 \pm 0.8	1.1
31	26.5 \pm 2.5	28.0	13.7 \pm 5.4	15.4	10.4 \pm 2.7	11.5	10.4 \pm 2.7	11.5	10.4 \pm 2.7	11.5	10.4 \pm 2.7	11.5	2.0 \pm 0.4	1.1	2.0 \pm 0.4	1.1
35	23.9 \pm 2.3	21.6	9.2 \pm 0.7	8.4	12.6 \pm 1.7	11.7	12.6 \pm 1.7	11.7	12.6 \pm 1.7	11.7	12.6 \pm 1.7	11.7	1.7 \pm 0.2	0.6	1.7 \pm 0.2	0.6
38	25.5 \pm 1.5	24.0	8.7 \pm 3.0	13.7	14.7 \pm 1.3	8.6	14.7 \pm 1.3	8.6	14.7 \pm 1.3	8.6	14.7 \pm 1.3	8.6	1.5 \pm 0.6	1.2	1.5 \pm 0.6	1.2
42	20.7 \pm 0.1	19.4	6.7 \pm 0.1	9.1	11.8 \pm 1.3	8.7	11.8 \pm 1.3	8.7	11.8 \pm 1.3	8.7	11.8 \pm 1.3	8.7	1.8 \pm 0.7	1.2	1.8 \pm 0.7	1.2
45	17.6 \pm 1.4	14.3	5.2 \pm 1.7	4.3	10.4 \pm 0.2	8.6	10.4 \pm 0.2	8.6	10.4 \pm 0.2	8.6	10.4 \pm 0.2	8.6	1.6 \pm 0.4	1.0	1.6 \pm 0.4	1.0

TABLE 50 EFFECTS OF CYTARABINE ON HAEMATOLOGY OF NORMAL PIGS

Group D - Saline Treated Controls										Group A - Low Dose										Group B - Medium Dose										Group C - High Dose										Hb - Means \pm 1 S.D																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
RBC - Means \pm 1 S.D										PCV - Means \pm 1 S.D										Group D										Groups A, B & C										Group D										Groups A, B & C																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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-28	7.33	+	0.29	7.39	+	0.36	0.36	+	0.02	0.36	+	0.03	12.1	+	0.5	12.3	+	0.8																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													

TABLE 50 Contd. EFFECTS OF CYTARABINE ON HAEMATOLOGY OF NORMAL PIGS

Group D - Saline Treated Controls		Group A - Low Dose		Group B - Medium Dose		Group C - High Dose		MCH - Means \pm 1 S.D	
Days From Start of Dosing	Group D	Groups A, B & C		Group D	Groups A, B & C		Group D	Groups A, B & C	
		A	B	C	D	A	B	C	D
-28	48.8 \pm 2.3	48.7 \pm 2.2	48.7 \pm 2.2	33.9 \pm 0.5	34.2 \pm 0.7	34.2 \pm 0.7	34.2 \pm 0.7	34.2 \pm 0.7	16.6 \pm 0.6
-21	50.6 \pm 0.8	50.0 \pm 1.4	50.0 \pm 1.4	33.2 \pm 0.4	33.4 \pm 1.2	33.4 \pm 1.2	33.4 \pm 1.2	33.4 \pm 1.2	16.7 \pm 0.5
-14	47.9 \pm 0.9	48.6 \pm 1.9	48.6 \pm 1.9	33.8 \pm 0.7	33.5 \pm 1.1	33.5 \pm 1.1	33.5 \pm 1.1	33.5 \pm 1.1	16.2 \pm 0.4
-7	45.7 \pm 2.6	45.8 \pm 2.0	45.8 \pm 2.0	34.5 \pm 0.8	34.8 \pm 1.0	34.8 \pm 1.0	34.8 \pm 1.0	34.8 \pm 1.0	16.0 \pm 0.6
0	47.9 \pm 1.5	47.6 \pm 1.8	47.6 \pm 1.8	33.5 \pm 1.2	34.2 \pm 1.2	34.2 \pm 1.2	34.2 \pm 1.2	34.2 \pm 1.2	16.3 \pm 0.6
Start of Dosing									
1	45.0 \pm 0.8	45.4 \pm 2.8	45.4 \pm 2.8	34.7 \pm 1.0	34.2 \pm 0.9	34.2 \pm 0.9	34.2 \pm 0.9	34.2 \pm 0.9	15.5 \pm 1.0
2	45.9 \pm 2.0	46.4 \pm 1.2	46.4 \pm 1.2	34.6 \pm 1.3	34.0 \pm 1.4	34.0 \pm 1.4	34.0 \pm 1.4	34.0 \pm 1.4	15.8 \pm 0.3
3	46.5 \pm 1.1	46.3 \pm 1.0	46.3 \pm 1.0	33.3 \pm 0.6	33.5 \pm 0.8	33.5 \pm 0.8	33.5 \pm 0.8	33.5 \pm 0.8	15.6 \pm 0.4
4	46.6 \pm 1.8	45.9 \pm 1.6	45.9 \pm 1.6	34.4 \pm 0.4	34.2 \pm 1.4	34.2 \pm 1.4	34.2 \pm 1.4	34.2 \pm 1.4	15.6 \pm 0.6
7	47.0 \pm 2.1	47.2 \pm 2.6	47.2 \pm 2.6	33.4 \pm 1.6	33.4 \pm 0.9	33.4 \pm 0.9	33.4 \pm 0.9	33.4 \pm 0.9	15.7 \pm 0.6
8	46.0 \pm 1.6	45.9 \pm 1.8	45.9 \pm 1.8	32.8 \pm 1.7	33.5 \pm 0.8	33.5 \pm 0.8	33.5 \pm 0.8	33.5 \pm 0.8	15.4 \pm 0.5
9	44.6 \pm 1.0	43.7 \pm 2.0	43.7 \pm 2.0	34.5 \pm 1.6	34.7 \pm 1.0	34.7 \pm 1.0	34.7 \pm 1.0	34.7 \pm 1.0	15.1 \pm 0.6
10	45.3 \pm 1.6	44.5 \pm 2.0	44.5 \pm 2.0	33.3 \pm 0.7	33.8 \pm 0.5	33.8 \pm 0.5	33.8 \pm 0.5	33.8 \pm 0.5	15.1 \pm 0.6
11	45.7 \pm 0.6	44.6 \pm 1.5	44.6 \pm 1.5	33.3 \pm 0.9	34.7 \pm 0.6	34.7 \pm 0.6	34.7 \pm 0.6	34.7 \pm 0.6	15.5 \pm 0.7
Dose Increased									
14	45.9 \pm 1.8	46.1 \pm 2.0	44.5 \pm 2.3	33.8 \pm 0.5	34.0 \pm 2.2	33.5 \pm 0.6	34.0 \pm 2.2	33.5 \pm 0.6	15.5 \pm 0.5
15	45.0 \pm 0.4	44.9 \pm 0.1	43.9 \pm 3.5	33.7 \pm 0.6	34.9 \pm 0.6	33.3 \pm 0.4	34.9 \pm 0.6	33.3 \pm 0.4	15.2 \pm 0.2
16	47.7 \pm 1.8	44.1 \pm 1.8	44.9 \pm 1.4	32.9 \pm 1.3	33.8 \pm 2.1	34.8 \pm 0.6	33.8 \pm 2.1	34.8 \pm 0.6	15.7 \pm 0.6
17	47.3 \pm 1.0	43.7 \pm 2.1	44.6 \pm 1.1	33.0 \pm 1.1	34.7 \pm 0.1	34.9 \pm 1.2	34.7 \pm 0.1	34.9 \pm 1.2	15.6 \pm 0.5
18	45.0 \pm 1.1	42.0 \pm 1.3	43.6 \pm 1.7	34.8 \pm 0.6	34.5 \pm 0.7	36.3 \pm 0.8	34.5 \pm 0.7	36.3 \pm 0.8	15.7 \pm 0.6
End of Dosing									
21	47.3 \pm 1.1	46.8	42.8	32.6 \pm 1.2	33.9	33.2	33.9	33.3	15.4 \pm 0.8
23									14.5
25	46.2 \pm 1.5	49.0	48.8	33.5 \pm 0.3	31.3	32.1	31.3	31.6	15.5 \pm 0.6
28	46.3 \pm 1.9	52.1	53.6	34.1 \pm 0.2	30.9	31.7	30.9	32.8	15.8 \pm 0.8
31	45.9 \pm 0.9	50.1	49.4	36.2 \pm 0.1	32.9	34.7	32.9	33.7	16.6 \pm 0.3
35	49.7 \pm 1.9	51.9	49.8	33.3 \pm 0	32.8	33.4	32.8	33.8	16.6 \pm 0.6
38	46.6 \pm 3.0	52.1	47.8	33.3 \pm 1.3	32.3	32.5	32.3	34.4	15.5 \pm 0.4
42	49.1 \pm 4.7	50.7	48.1	32.3 \pm 1.1	32.0	32.9	32.0	32.4	15.9 \pm 0.9
45	49.4 \pm 0.6	52.4	50.8	32.3 \pm 0.4	31.3	32.4	31.3	33.1	15.9 \pm 0
									15.9 \pm 0
									17.0
									15.5
									15.4
									15.4
									16.7
									17.0
									16.2
									16.4
									16.9

treated pigs in weeks 1 and 2, and of the controls and low, medium and high-dose groups in week 3. As with the white cells, RBC counts, PCV and Hb were decreased in the treated animals, but the effect was not seen until the beginning of the second week of treatment.

Figures 28 and 29 compare mean Hb, RBC counts and PCV respectively of all the treated animals compared with the controls. As before the maximum effect was observed in week 3 and it was not dose-related, all treated groups being similarly affected (See Table 50). In the remaining animals after dosing was stopped, RBC counts, PCV and Hb remained lower than the mean values of the controls for a further 10 days, and then rapidly recovered. There was no treatment-related effect on MCV, MCHC or MCH, but MCV was slightly increased in the treated group during the recovery period, especially on day 28 (52.0 ± 1.6 fl compared with 46.3 ± 1.9 fl in the control group).

Figure 29 shows mean thrombocyte counts of the treated animals compared with the controls. As with the white and red cell pictures, thrombocyte counts were depressed in the treated animals, but this effect did not become obvious until the 3rd week. Table 51 shows mean counts for low -medium-and high-dose groups in week 3, compared with the controls and once again, the effect was not dose-related, being similar in all treated groups. When treatment was stopped there was rapid recovery in the remaining animals, but before the improvement occurred

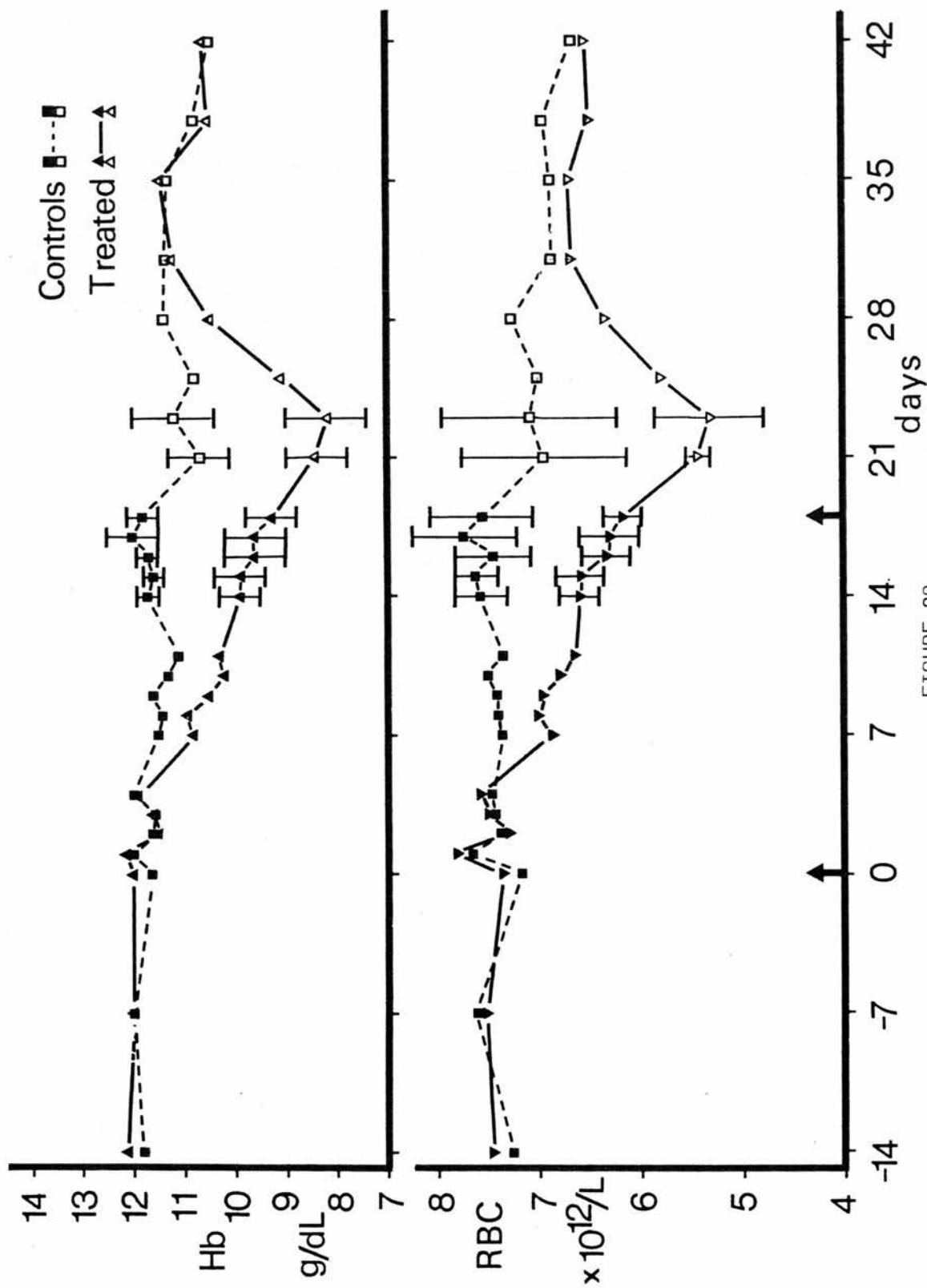


FIGURE 28

Mean Hb and RBC counts of six normal pigs treated with cytarabine compared with mean values of four saline-treated controls. Arrows indicate beginning and end of treatment. Open symbols after half of the pigs were killed.

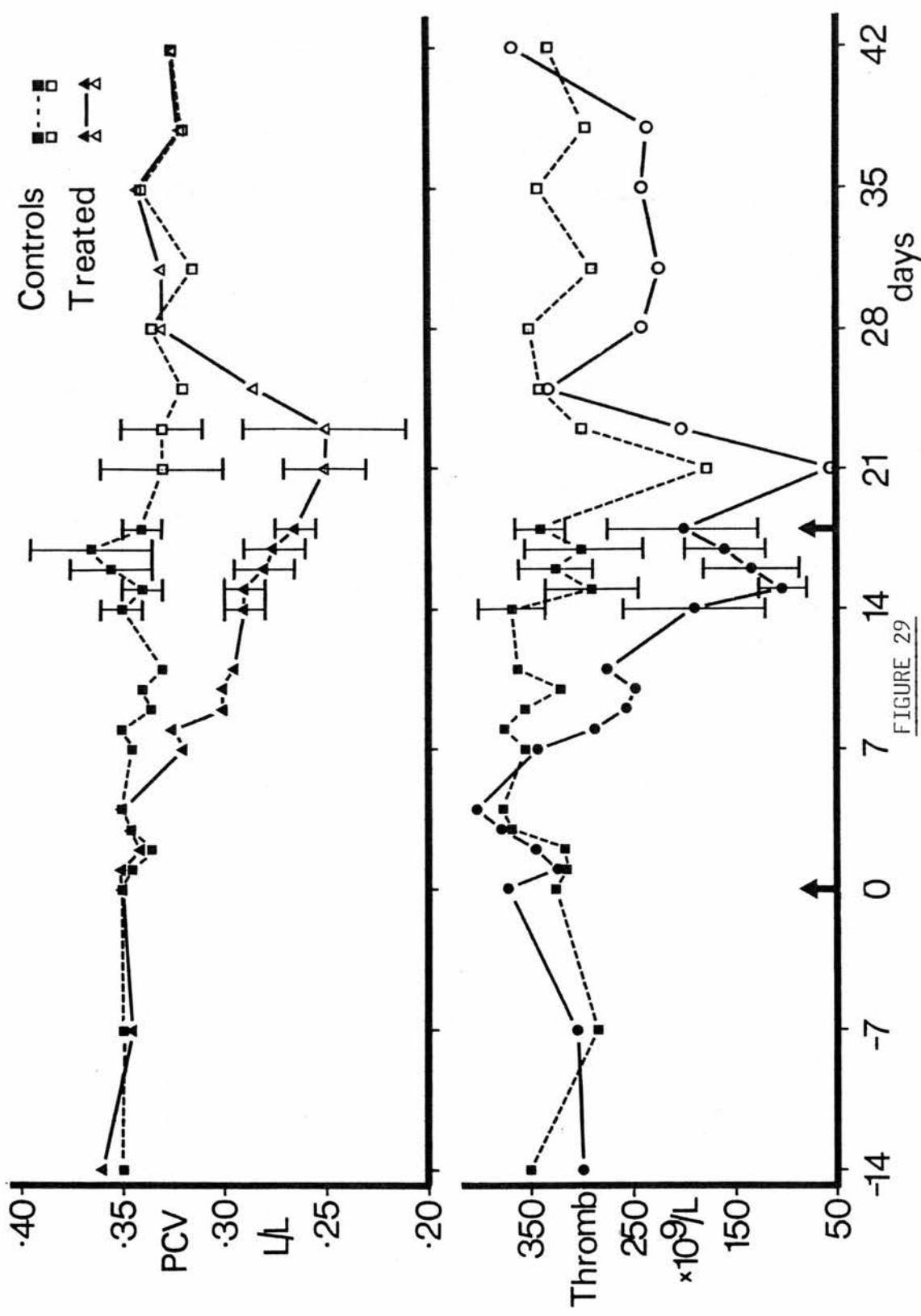


FIGURE 29

Mean PCV and thrombocyte counts of six normal pigs treated with cytarabine compared with mean values of four saline-treated controls. Arrows indicate beginning and end of treatment. Open symbols used after half of the pigs were killed.

TABLE 51
CYTARABINE IN NORMAL PIGS

Mean Thrombocyte			Counts			$\pm 1 \text{ S.D.} \times 10^9/\text{L}$			
Days from Start of Dosing	Group D			Treated Groups					
	Controls			A	+B		+ C		
- 28	472	\pm	50		361	\pm	144		
- 21	309	\pm	56		311	\pm	49		
- 14	350	\pm	86		299	\pm	49		
- 7	286	\pm	41		306	\pm	57		
0	327	\pm	14		372	\pm	44		
Start of Dosing									
1	316	\pm	35		321	\pm	49		
2	317	\pm	31		345	\pm	51		
3	369	\pm	29		378	\pm	88		
4	377	\pm	26		403	\pm	122		
7	354	\pm	16		343	\pm	94		
8	374	\pm	58		287	\pm	69		
9	357	\pm	91		258	\pm	64		
10	322	\pm	22		248	\pm	51		
11	362	\pm	49		275	\pm	60		
Dose Increased									
D				A-	B-		C-		
				Low Dose	Medium Dose		High Dose		
14	368	\pm	32	157	\pm	86	178	\pm	64
15	291	\pm	45	103	\pm	33	136	\pm	21
16	325	\pm	36	105	\pm	13	131	\pm	2
17	299	\pm	58	154	\pm	62	187	\pm	26
18	340	\pm	24	243	\pm	123	161	\pm	14
End of Dosing									
*									
21	178	\pm	168	29			81	54	
23	300	\pm	52	194			248	167	
25	340	\pm	76	396			359	247	
28	350	\pm	103	237			269	220	
31	288	\pm	37	249			191	236	
35	342	\pm	163	164			329	229	
38	295	\pm	34	198			245	263	
42	332	\pm	82	316			465	321	
45	265	\pm	57	394			346	352	

* Two controls and one pig from each treated group destroyed.

very low counts were recorded on day 21 (mean of $55 \pm 26 \times 10^9/l$). However, there were no signs of thrombocytopenic purpura.

Serum Enzymes

SGOT and serum alkaline phosphatase levels in individual animals are shown in Tables 33 and, 34 in Appendix IV. All values recorded were on the low side of the normal range. There were no differences between groups which were likely to be caused by the treatment, though serum alkaline phosphatase levels tended to be lower in the treated pigs than in the controls.

Blood Urea (See Appendix IV, Tables 21 to 30)

All blood urea measurements before, during and after treatment were within the normal range.

Serum Proteins (See Appendix IV, Tables 31 and 32).

Total protein and albumin were measured from serum as described in the "Methods" section and total serum globulin levels were obtained by difference.

Table 52 shows means ± 1 S.D. of the albumin and globulin values recorded just before, during, and just after the treatment period.

It can be seen that there was a slight progressive rise in serum albumin during treatment, though only in Group B on day 18 are the standard deviations narrow enough to suggest a real effect. The albumin levels fell again in all groups by day 25.

There was a fall in globulins in all groups even

TABLE 52

CYTARABINE: NORMAL PIGS

SERUM PROTEINS - MEAN \pm 1 S.D. (g/l)

Start of Dosing	SERUM ALBUMIN			SERUM GLOBULINS (BY DIFFERENCE)		
	GROUP D	TREATED GROUP			GROUP D	TREATED GROUP
		GROUP A	GROUP B	GROUP C		
0	33.2 \pm 3.7		32.4 \pm 1.7		29.5 \pm 0.8	28.4 \pm 3.9
Start of Dosing						
4	33.7 \pm 1.2		37.1 \pm 2.9		21.9 \pm 2.4	19.3 \pm 0.9
11	34.5 \pm 3.9		37.9 \pm 4.1		24.6 \pm 5.2	21.9 \pm 3.0
		GROUP A	GROUP B	GROUP C		
18	33.5 \pm 1.1	38.3 \pm 6.2	39.0 \pm 1.3	35.9 \pm 1.1	27.5 \pm 2.8	22.6 \pm 0.1
End of Dosing						
25	26.2 \pm 0.9	28.4	29.6	28.4	30.1 \pm 2.8	27.3
					27.8	27.0

in the control group at the beginning of the treatment period, but the decrease was slightly greater in the treated group. On day 18, globulins in group B were particularly depressed compared with the control group.

By day 25, globulins had increased again in all groups.

POST-MORTEM RESULTSControls

309 and 305 - killed at the end of treatment.

These were normal carcasses and the viscera showed no abnormalities.

The thymuses were composed of moderately large firm fawn lobules.

The bone marrow of the femurs was red and active in the proximal half of the shaft but floated in formalin.

At the site of removal of the right inguinal lymph nodes there was in each a small red ulcerated nodule which on cut surface extended into the underlying tissue like a healed sinus. The subcutaneous fat showed ill-defined areas of fibrosis with yellowish firm nodules from which no pus could be expressed.

All carcass and visceral lymph nodes were of normal size and showed a nodular 5mm white cortex surrounded by a variable 1mm brownish medulla.

304 - killed at the end of the experiment.

No abnormalities were detected in carcass or viscera, except in the inguinal region where the lymph nodes had been removed. On the right side under the skin there was an irregular mass of fibrous tissue about 10mm thick, and on the left side a mass about 25mm thick with a soft yellowish granuloma.

308 -

Similar to 304, except that the areas of fibrosis

were smaller and less marked.

Low-dose pigs

312 - killed at the end of treatment.

This normal carcass resembled those of 309 and 305.

307 - killed at the end of treatment.

The carcass and viscera were normal, except for a 2mm raised red nodule on the mucosa in the mid-length of the caecum, and fibrous masses in the inguinal region. The stomach contents were very fluid.

Medium-dose pigs

310 - killed at the end of treatment.

This normal carcass resembled those of 309 and 305.

300 - killed at the end of the experiment.

No abnormalities were detected except for very slight fibrosis at the sites of the removed inguinal lymph nodes.

High-dose pigs

306 - killed at the end of treatment

This normal carcass resembled those of 309 and 305.

298 - killed at the end of the experiment.

No abnormalities were detected except for a fibrous reaction at the site of the left inguinal lymph node which had a central yellow core of pus.

The stomach contents were very fluid.

Relative weights of lymph nodes and organs

It can be seen from Table 53 that the relative weights of the mesenteric and colonic nodes of medium dose pigs 310 and 300 were considerably less than those of the control pigs, even though 300 was killed 4 weeks after treatment was stopped. The same group of nodes in the high-dose pig, 306, however, were heavier than in the controls.

The thymuses of the treated pigs 312, 310 and 306 were very much smaller than those of the controls, particularly pronounced once again in medium-dose pig 310. The thymuses of the treated pigs killed 4 weeks later were also slightly smaller than those of the controls.

TABLE 53

CYTARABINE IN NORMAL PIGS - RELATIVE WEIGHTS OF LYMPH NODES AND ORGANS (cg/kg body weight)

DOSE LEVEL	PIGS KILLED AT END OF TREATMENT				PIGS KILLED AT END OF EXPERIMENT			
	CONTROLS	LOW	MEDIUM	HIGH	CONTROLS	LOW	MEDIUM	HIGH
	305	309	312	310	304	308	300	298
Lymph Nodes :-								
Mesenteric and								
Colonic	327	315	317	281	385	382	313	353
Gastric and								
Splenic	24	40	38	34	46	63	50	59
Bronchial Group	13	17	22	22	13	20	22	23
Total Splanchnic	364	372	377	337	444	465	385	435
Head Group	31	35	29	31	32	32	26	33
Iliac Group	7	8	8	9	8	10	13	9
Prescapular	7	4	3	4	5	4	5	6
Precrural	3	3	3	4	5	5	4	5
Inguinal	20	20	20	41	-	-	-	-
Popliteal	2	1	6	1	2	1	1	3
Total Superficial	70	71	69	90	52	52	49	56
Thymus	126	191	42	23	141	144	114	94
Spleen	200	278	259	333	205	324	250	294
Liver	2440	2972	3222	2923	3154	3324	3063	3765
Kidney	180	194	248	241	231	250	234	294
Adrenal	5	4	5	5	5	5	6	6

DISCUSSION

The results of this experiment indicate that subcutaneous, twice daily administration of cytarabine produced no clinical signs of toxicity, other than slight weight loss. The haematology results indicated a progressive, but apparently not dose-related, bone marrow depression with all types of WBC, RBC and thrombocyte counts affected. There was a slight fall in serum globulins and a rise in serum albumin. These effects were temporary and were reversed in the surviving animals when cytarabine administration was stopped.

The haematological effects are similar to those which have been recorded in man (Kremer, 1975). These effects, and the serum protein effects were most pronounced in treated Group B. According to Kremer, the leucopenia produced in man is due primarily to granulocyte depression as lymphocyte counts are barely affected. Although absolute lymphocyte counts in the pigs were depressed, this experiment showed that the neutrophils were more severely affected. Kremer (1975) also stated that when thrombocyte counts recover in man, there is often a significant "overshoot" with counts reaching $1000 \times 10^9/l$. This was not observed in the pigs.

The post-mortem evidence of thymus suppression was not associated with an effect on skin delayed hypersensitivity reactions. However, this agreed with Kremer (1975), when he stated that cytarabine prevents the

induction of delayed hypersensitivity to dinitrochlorobenzene but it has no effect on already established hypersensitivity.

SGOT and alkaline phosphatase levels in the pigs were unaffected. They were measured because there have been occasional reports in man of the occurrence of abnormal liver function tests with cytarabine. However according to Kremer (1975) these have been rare, and not definitely established as due to the drug.

Cytarabine Therapy

Materials and Methods

Animals and pretreatment procedures

Four pigs were used for the therapy trial. All were cases of lymphosarcoma which were diagnosed before 8 weeks of age. Three were entire males and one was a female. The female and one male were littermates, and the other two males were also littermates. The first pair were almost 4 weeks older than the second pair. Both pairs were moved to the experimental animal house at 8 weeks of age. They were then kept in isolation in individual pens and allowed to become accustomed to their surroundings for several weeks. Management was as described in "General Methods".

During this period, all four animals became ill and required treatment. Female 4243 and male 4248 became febrile and dyspnoeic with harsh dry lung sounds. Pig 4243 had impetigo. Intramuscular injections of chloramphenicol were administered for 5 days. Rapid improvement occurred, but lung sounds in 4248 remained abnormal for some weeks after this episode. Males 317 and 318 developed severe diarrhoea soon after being moved to the animal house. Treatment with neomycin sulphate and "Streptaquine" mixture brought about temporary improvement but treatment had to be repeated on three occasions before the faeces returned to normal consistency.

Staging laparotomy was carried out 3½ weeks before cytarabine treatment was started, as described in the "Methods" section, pages 81 to 84. The diagnosis of lymphosarcoma was confirmed and all four animals had widespread abdominal involvement with gross enlargement of the spleen and gastric and mesenteric lymph nodes. The livers were pale and mottled. There was at least 1 litre of fluid in the abdomen of 318. This was clear and reddish-brown and formed a soft clot on standing. There was no evidence of peritonitis. Abdominal fluid was present in all lymphosarcoma pigs which were subjected to laparotomy, especially the untreated cases, but was rarely seen in large amounts at post-mortem. Two pieces of mesenteric lymph node were removed, one for histopathological examination and one for DCK estimation. The former was placed in 10% formalin and the latter was wrapped in aluminium foil and deep frozen. No tissue was removed from pig 317 because the splenic capsule developed a small rupture while being handled. Haemorrhage was not severe but it was thought to be unwise to continue the operation.

Recovery from laparotomy was uneventful except for a breakdown of some of the internal sutures in pig 4243, resulting in a large hernia. Since this did not appear

to inconvenience the animal, and healing of the skin incision was rapid, repair was not attempted.

One week after surgery, all the pigs were sensitised with DNFB, and skin tests were carried out 2 weeks after sensitisation.

Weekly weighing and blood-sampling commenced 4 weeks before the start of treatment and detailed clinical examinations with rectal temperature recording and lymph node measurements 2 weeks before, after the laparotomy incisions were healed. Haematology samples were taken and also clotted samples for estimation of serum total protein, albumin, SGOT and alkaline phosphatase. Fitness, appetite and any abnormal behaviour were noted daily, also the state of urine and faeces.

Treatment

At the start of treatment, 4243 and 4248 were 6 months old, and 317 and 318 were 5 months old.

As in the experiment using normal pigs the preparation was "Cytosar" and the drug was prepared for injection as previously described.

The pigs were heavier than the normal animals used in the trial but as all were in very poor condition with large amounts of tumour, it was decided to start with exactly the same cytarabine dosage schedule as had been used in the previous experiment and to use the same in all four pigs, i.e. 25mg subcutaneously twice daily for 5 days/week. After 2 weeks on this dose level, it was doubled to 50mg

twice daily for 5 days. As in the trial using normal pigs, the drug was administered first thing in the morning and in the late afternoon.

During the treatment period, weekly weighing and clinical examinations were continued. Blood samples for haematology were taken daily, 5 days/week. A larger sample was taken once weekly for serum proteins and SGOT, and blood urea estimations were carried out twice weekly on Mondays and Fridays.

Skin tests were repeated during the last week of treatment.

At the end of the 3 weeks of treatment two of the four pigs, 4243 and 318 were destroyed by pentobarbitone overdose and autopsied. Another laparotomy was carried out on pigs 4248 and 317, so that the effects of the drug could be assessed in all four pigs. Weekly weighing and clinical examinations were continued for 4 weeks after the end of treatment. Blood sampling for haematology was carried out three times weekly on Mondays, Wednesdays and Fridays during this time. At the end of the 4 weeks, these pigs showed no remaining effects of cytarabine treatment. As the number of new lymphosarcoma cases available for drug trials was very small at that time, they were not destroyed and were given a different drug, therefore autopsy results are not available for these two animals.

RESULTS

Clinical Examinations (See Appendix IV)

Pig 4243 - This female was rather depressed during the 1st week of treatment. It had a cough and its mucous membranes and skin were extremely pale. The impetigo noted during the pretreatment period had returned and its appetite was poor. The rectal temperature was within the normal range and auscultation of the chest did not reveal any abnormality. Benzathine penicillin was administered intramuscularly. During the 2nd and 3rd weeks of treatment, there was a marked improvement in the pig's condition and it became quite difficult to handle. The circumference of its abdomen decreased by 4cm from the beginning to the end of treatment even though its appetite had improved. This decrease occurred during the 3rd week of treatment.

It had sufficient subcutaneous fat, despite the advanced stage of the disease, to make accurate measurement of the precrural lymph nodes impossible. The size of the inguinal lymph nodes (see Table 54) decreased by 21%, again mainly during the 3rd week of treatment.

This pig was destroyed at the end of the 3rd week.

Pig 4248 - This male was also quiet and rather depressed at the beginning of treatment and its appetite was poor. Its rectal temperature stayed within the normal range. There was no change in its general condition during the first 2 weeks of therapy, but during the

3rd week, it improved considerably. The circumference of its abdomen decreased by 5cm during therapy, from 97cm to 92cm, again despite an improvement in appetite.

The size of the inguinal lymph nodes decreased by 34% and the precrurals by 31% when the measurements of week 3 are compared with those of week 0. (See Table 54).

After the second laparotomy, the pig was very depressed, and it only regained its appetite 10 days later. It lost 6.5kg during this time, which interfered with the results shown in Table 55. Although rectal temperatures were still within the normal range, antibiotic cover was thought to be advisable and intramuscular injections of ampicillin were given for 7 days after surgery. After this time, it developed severe diarrhoea, but its appetite had improved slightly and so it was given neomycin sulphate orally with kaolin. Healing of the incision was uneventful, and the diarrhoea stopped.

By 4 weeks after the end of cytarabine therapy, the circumference of its abdomen had increased to 99.5cm. The inguinal and precrural lymph nodes increased in size rapidly by 66%, when the measurements of week 7 are compared with those taken at the end of week 3. (See Table 54).

After this, 4248 was given a different drug.

Pig 317 - This male was well and active throughout the treatment period, apart from a mild attack of diarrhoea at the end of the 1st week which responded to oral neomycin sulphate, administered for 5 days. Its appetite was moderately good, and was unaffected during the time it had diarrhoea. Its rectal temperature stayed within the normal range.

The circumference of the abdomen increased from 87.5cm to 89.5cm during treatment. The size of the inguinal lymph nodes decreased only 6% during the 3 weeks of treatment, but decreased a further 29% during the 1st week after treatment was stopped. The precrural nodes decreased by 32% during therapy and did not decrease any more after it was stopped (See Table 54).

Five days after the second laparotomy, 317 again developed diarrhoea. The rectal temperature was still within the normal range, but it would not eat and was very depressed. Ampicillin was injected intramuscularly for 3 days and there was a rapid improvement. This treatment was repeated 2 weeks later when the pig was noticed to be off its food once again. On this occasion, the faeces were not so fluid, but its rectal temperature was 40.8°C. Healing of the laparotomy incision was rapid.

By 4 weeks after the end of treatment, its abdominal measurement had increased slightly to 90.5cm. Its inguinal and precrural lymph nodes had increased in size

again, having similar measurements to those obtained just before the start of cytarabine treatment.

Like 4248, this animal was then given a different drug.

Pig 318 - This male was in poor body condition and very pale and anaemic-looking at the start of treatment. Its appetite was fairly good. Its condition did not improve during treatment, and it suffered from diarrhoea almost constantly. A 5 day course of neomycin sulphate in its food brought about a slight improvement but at the end of the 2nd week it was very sick. It had no appetite and its rectal temperature had risen to 42.2°C. Chloramphenicol was administered intramuscularly for 4 days. The faeces became more normal but its appetite remained very poor and it would only eat green vegetables. There was a slight improvement during the 3rd week but when it was destroyed at the end of the week it had severe diarrhoea once again.

The abdominal measurements fluctuated, probably due to variation in intestinal contents, but there was an overall increase of 1.5cm during the 3 weeks of cytarabine treatment.

The inguinal lymph nodes decreased in size by 33% from the beginning to the end of treatment, and the precrurals by 39% (See Table 54).

Weight gains

The results of body weight gains over 3-weekly periods, pretreatment, during treatment and after treatment,

TABLE 54

CYTARABINE THERAPY

Lymph node measurements - mean products of lengths and breadths of inguinal and precrural nodes (mm²).

Week	PIG NO. 4243		PIG NO. 4248		PIG NO. 317		PIG NO. 318	
	Inguinal	Precrural	Inguinal	Precrural	Inguinal	Precrural	Inguinal	Precrural
-1	1845	Too fat	3056	1001	2089	692	1984	677
0	2161	"	2584	781	2018	728	2070	667
Start of Dosing								
1	1914	"	2208	587	1930	760	2160	810
2	2143	"	2215	787	1925	751	1736	604
3	1706	"	1711	536	1892	497	1394	408
End of Dosing								
4	DEAD		1751	543	1348	528	DEAD	
5			2048	611	1978	683		
6			2102	674	2106	638		
7			2847	891	2037	684		

TABLE 55

CYTARABINE THERAPY - BODY WEIGHT GAINS (kg)

PIG No.	Pretreatment (3 weeks)	During treatment (3 weeks)	After treatment (3 weeks)
4243	8.5	2.5	Dead
4248	-1	6.5	-2.5
317	8	3	-0.5
318	5	1.5	Dead

are shown in Table 55.

During the pretreatment period, the weight gains achieved by pigs 4243, 317 and 318 were fairly good, and in all three weight gains during treatment were depressed. The only pig to show an improvement was 4248, which lost weight during the pretreatment period because of a respiratory infection.

Both 4248 and 317 which were left alive lost weight during the post-treatment period as a result of illness.

TABLE 56

CYTARABINE THERAPY

DNFB Skin-Test Results

Pig No.	At sensitisation	Pre-treatment	Last week of treatment
4243	2	3	2
4248	3	2	3
317	3	3	1
318	2	3	1

DNFB Skin-Test Results

The pretreatment reaction scores, shown in Table 56 were quite high for lymphosarcomatous pigs, three out of the four pigs showing marked erythema and skin thickening with vesicle formation (3). During the last week of treatment, one pig, 4248, had an increased reaction

score, but those of the other three pigs were reduced. It appears, therefore, that the cytarabine treatment may have caused a slight suppression of cell-mediated response in three out of the four pigs.

Unfortunately, the two pigs left alive at the end of the experiment were not retested.

Haematology

See Tables 36 to 43, Appendix IV for results from the individual pigs.

Table 57 shows the means \pm 1 S.D. of WBC and absolute neutrophil and mononuclear cell counts of the four pigs, and after day 18, the two pigs which had not been destroyed.

During each of the three 5 day periods of treatment, the lowest WBC counts were recorded on days 3, 10 and 17. There was a slight overall reduction in mean WBC counts during treatment but the standard deviations were wide. If the absolute figures for each pig are considered individually, there was an overall increase in WBC counts in pigs 4243 and 4248 from day 0 to day 18, and a decrease in pigs 317 and 318 during the same time. See Table 58. The mononuclear cell counts show very similar changes, with a mean decrease, from 19.3 ± 8.2 to $14.7 \pm 3.9 \times 10^9$ /litre. The effect on morphologically abnormal cells varied. There were still many in blood smears from pig 4248 at the end of treatment but very few were present in those from pig 318.

TABLE 57

CYTARABINE THERAPY

MEANS \pm ISD OF WBC, ABSOLUTE NEUTROPHIL AND MONONUCLEAR
CELL COUNTS, $\times 10^9$ /LITRE :

DAYS FROM START OF DOSING	WBC			NEUTROPHILS			MONONUCLEAR CELLS		
- 20	20.7	\pm	5.2	7.6	\pm	6.3	13.0	\pm	3.0
- 14	27.4	\pm	4.5	7.8	\pm	1.7	19.3	\pm	4.0
- 7	30.9	\pm	4.2	5.1	\pm	2.7	25.3	\pm	4.0
0	26.0	\pm	10.7	6.6	\pm	2.8	19.3	\pm	8.2
Start of Dosing									
1	23.0	\pm	10.4	6.5	\pm	3.5	16.3	\pm	7.2
2	22.9	\pm	9.5	5.2	\pm	2.1	17.6	\pm	7.9
3	20.5	\pm	6.5	6.4	\pm	2.2	14.1	\pm	6.0
4	22.3	\pm	7.1	5.6	\pm	4.6	16.6	\pm	7.6
7	25.3	\pm	3.1	4.7	\pm	0.9	20.5	\pm	3.8
8	20.4	\pm	2.5	4.5	\pm	1.7	15.8	\pm	4.0
9	21.4	\pm	4.1	3.2	\pm	1.3	18.2	\pm	5.2
10	16.7	\pm	6.3	4.1	\pm	1.8	12.6	\pm	7.6
11	18.6	\pm	4.6	4.6	\pm	1.0	13.7	\pm	5.4
Dose Increased									
14	21.7	\pm	5.4	4.2	\pm	1.4	17.2	\pm	5.2
15	16.8	\pm	2.8	3.0	\pm	1.8	13.6	\pm	4.6
16	16.9	\pm	2.1	3.3	\pm	1.5	13.4	\pm	3.8
17	15.1	\pm	2.0	2.3	\pm	1.7	12.3	\pm	2.4
18	17.6	\pm	5.1	2.6	\pm	2.0	14.7	\pm	3.9
End of Dosing ⁺									
21	21.3	\pm	10.1	4.6	\pm	3.5	16.6	\pm	6.7
23	24.9	\pm	11.9	9.8	\pm	4.3	15.1	\pm	7.6
25	24.0	\pm	0.8	10.0	\pm	1.0	13.8	\pm	1.6
28	27.5	\pm	7.6	12.7	\pm	7.4	14.6	\pm	0
30	27.6	\pm	2.2	9.6	\pm	3.3	17.7	\pm	1.4
32	22.3	\pm	0.4	7.5	\pm	0.9	14.7	\pm	0.7
35	23.9	\pm	3.3	5.6	\pm	0.9	17.9	\pm	2.1
37	24.3	\pm	4.0	8.5	\pm	1.0	15.7	\pm	2.7
39	30.0	\pm	0.1	6.6	\pm	1.7	23.2	\pm	2.0

+

Two out of the four pigs destroyed.

Absolute neutrophil counts at the end of treatment were decreased in three out of the four pigs (See Table 58).

As with the mononuclear cells, the effect on pigs 317 and 318 was more pronounced. After therapy was discontinued, there was a rapid increase in neutrophil counts from day 21 to day 28, followed by a gradual return to pretreatment levels.

The effects of cytarabine on the red cell picture also varied between pigs (See Appendix IV).

Female 4243 showed a sudden deterioration before the start of treatment. During the first 5 days of cytarabine administration, the deterioration continued, but during the following 2 weeks of therapy, the RBC count rose from 3.18 to $5.02 \times 10^{12}/l.$, the PCV rose from 0.185 to 0.32 l/l and Hb rose from 7.4 to $10.2g/dl.$ There was an overall increase in MCV in this animal from 53.8 fl on day 0 to 63.7 fl on day 18 and a slight fall in MCHC from 35.2 to 31.9 g/dl.

Pig 4248 showed an initial improvement in red cell picture during treatment, but there was a deterioration during each of the following 2 weeks resulting in a fall in RBC count from $4.93 \times 10^{12}/l$ on day 0 to $4.68 \times 10^{12}/l$ on day 18. There was, however, a rise in PCV from $0.2551/l$ to 0.30 l/l, therefore the calculated MCV rose markedly from $51.7fl$ to $64.1fl.$ There was little overall change in Hb and a slight fall in MCHC. After the end of treatment, the values of RBC, PCV and Hb recorded

TABLE 58

CYTARABINE THERAPY

Changes in WBC picture of individual pigs during the treatment period (day 0 to day 18).

Pig No.	WBC $\times 10^9/\text{litre}$	Mononuclear cells $\times 10^9/\text{litre}$	Neutrophils $\times 10^9/\text{litre}$
4243	+ 5.6	+ 6.4	- 1.7
4248	+ 1.2	+ 1.3	No change
317	- 12.2	- 5.2	- 7.2
318	- 28.3	- 21.1	- 7.3

on day 25 were almost as high as those of a normal pig. However, the animal was ill with diarrhoea at this time, therefore it seemed likely that this indicated dehydration, particularly as the calculated MCV value was still high at 62.2fl and the blood urea level was 10.35mmol/l, slightly higher than normal.

Two and a half weeks after the end of treatment, RBC, PCV and Hb had genuinely improved to $5.95 \times 10^{12}/\text{l}$, 0.301/l and 10.5 g/dl respectively, and the MCV value indicated that the red cells were smaller at 50.4fl. After this time the pig again became gradually more anaemic.

Pig 317 showed a progressive deterioration in its red cell picture throughout the period of cytarabine administration. The RBC count decreased from 5.48 to $3.96 \times$

$10^{12}/l$, PCV from 0.27 to 0.22 l/l and Hb from 9.5 to 7.2 g/dl. MCV increased only slightly from 49.3 to 55.6 fl. Following the end of treatment there was a slight improvement, but after day 28 the pig once again became gradually more anaemic.

Pig 318 was the most anaemic of the four at the start of treatment, and apart from a slight improvement on day 14, its red cell picture continued to deteriorate. The RBC count fell from 3.50 to $2.62 \times 10^{12}/l$, PCV from 0.23 to 0.17 l/l and Hb from 7.7 to 5.6 g/dl during therapy. There was little overall change in the calculated indices in this animal but the MCV showed that the red cells were large (64.9 fl).

Thrombocyte counts fluctuated widely throughout the treatment period, but were mostly between 100 and $200 \times 10^9/l$. Low counts were frequently obtained from pig 4243 but these were always associated with severe clumping of thrombocytes. On occasions, this was due to delay in obtaining the samples, as this animal was rather difficult to handle, but it also occurred when there were no sampling problems. High counts (up to $300 \times 10^9/l$) were recorded in pig 4248 after the end of therapy. See Appendix IV).

Serum Enzymes

Serum alkaline phosphatase and SGOT levels in the individual pigs are shown in Table 44, Appendix IV.

Serum alkaline phosphatase levels were within or

lower than the normal range for pigs of this age throughout the experiment.

SGOT levels fluctuated, but in general these were also within the normal range. Pig 4243 had a slightly increased level on day 0, but it decreased by day 4.

Blood Urea

See Appendix IV, Tables 40 to 43).

Slightly increased blood urea levels were recorded in pig 317 on day 18, the last day of treatment, (11.29 mmol/l) and in pig 4248 after the end of treatment on day 25 (10.35). There was no clinical indication of a reason for the increase in 317, but on day 25, 4248 was dehydrated with severe diarrhoea. Otherwise blood urea levels were within the normal range.

Serum Proteins

See Appendix IV, Tables 45 and 46).

All four pigs had fairly low serum albumin levels before treatment started. By subtraction of albumin from total protein levels, high total globulin levels were calculated, particularly in pigs 4248 and 317.

During treatment, there were slight fluctuations but the overall change in serum albumin was a decrease, particularly in pig 318. This animal had a marked increase in serum globulins at the end of treatment, but the other pigs showed no changes in globulins which could be attributed to the treatment. From day 0 to day 18,

the albumin/globulin ratios fell in all four animals, particularly in pig 318 (0.53 to 0.24).

During the post treatment period, there was little change in the albumin or globulin levels in pig 317, but 4248 showed a rise in serum albumin and a fall in globulins, despite apparent progression of the tumour.

Post-Mortem Results

Pig 4243 - killed at the end of treatment.

This was a well-developed, well-nourished carcass. The head and neck including the brain, showed no abnormality.

The thymus consisted of small, fawn lobules lying in fat and connective tissue. No thymus was found in the chest.

There was a little clear fluid in the pericardial sac but the heart was normal.

The tip of the left cardiac and the whole of the right cardiac lobes of the lungs showed depressed nodular consolidation. The cut surface was collapsed and cellular with a little pus in the bronchi.

The liver showed adhesions to the left anterior flank and to the spleen where laparotomy had been carried out. The adhesion was relatively easily broken down. Liver lobulation was not distinct and the organ was of normal colour.

There was a 15cm pendulous umbilical hernia. On cut surface it had a 5cm thick fibrous and oedematous

wall. Some coils of the colon were attached by firm fibrosis between the serosa and the inner lining of the hernia but there was no obvious obstruction of the colon.

The stomach contained a little bedding. The oesophageal region showed slight nodular yellow crustiness but no ulceration.

The duodenum was bile-stained and was not thickened. The mucosa of small intestine, caecum and colon was normal. Peyer's patches were just visible as thickened areas in the mucosa.

The spleen was large but there were no nodules along the hilus. The cut surface showed engorgement and ill-defined 2-3mm white pulp foci throughout.

The kidneys showed a few ill-defined 1mm white foci in the cortex.

The adrenals, uterus and bladder were normal. The ovaries showed numerous clear, red 3-4mm cysts.

The bone marrow of the femur was red and white mottled. It was cellular and sank in formalin.

All lymph nodes were nodular, fawn and soft, with no medulla except at one pole of the prescapular and in the mesenterics, iliacs and inguinals. The bronchials, mesenterics, gastrics, iliacs and inguinals showed a few small cysts.

Pig 318 - killed at the end of treatment.

This was a rather small pig but there was plenty of fat present. The blood was very thin and watery.

The head and neck, including brain and tonsils, showed no abnormality.

There was a little clear fluid in the chest cavity and pericardial sac. All major lymphatic vessels were full of lymph and the lymph nodes were oedematous.

The thymus in the neck consisted of small, fawn, rather wet nodules. No thymus was found in the chest.

Lungs, heart, adrenals, kidneys and bladder were normal.

The liver was large but the edges were sharp and it was of normal colour. The lobulation was not distinct and there were no focal lesions.

The spleen was large and there were adhesions to the flank at the site of the laparotomy incision in the left anterior mid flank region. There were 2-3cm diameter sessile nodules along the hilus and occasional smaller ones along the edges. The cut surface was congested, with ill-defined 1-2mm white pulp foci. The nodules were soft and cellular, red and white.

The testes and epididymis were small but normal. On cut surface, the testis showed 5mm red foci of haemorrhage.

The oesophageal region of the stomach showed yellow crustiness but no ulceration. The duodenum was slightly thickened and bile-stained.

The small intestine contained a little fluid and the mucosa was normal. The Peyer's patches were slightly

thickened. Many of the loops of the small intestine were caught up in the fibrinous reaction noted around the spleen and there may have been some restriction of movement in those loops, though they were not more congested than elsewhere.

The mucosa of caecum and colon was normal but the contents of the colon were rather fluid.

The bone marrow of the whole length of the femur was red and white mottled, cellular and sank in formalin.

All lymph nodes showed a firm, fawn flat field of tissue, with no medulla except in the iliacs, inguinals and splenic. In the splenic, the medulla was haemorrhagic. The bronchial and mesenteric nodes contained small clear cysts.

Relative Lymph Node and Organ Weights

Table 59 shows the relative weights from pigs 4243 and 318 killed at the end of treatment, compared with those from three normal and 14 untreated lymphosarcoma pigs of similar age. It can be seen that although the relative lymph node weights from the treated pigs are reduced compared with the mean values from untreated cases, they are still within the range of weights from untreated cases, and much larger than the range of relative weights from normal pigs. Although the total weights of superficial nodes are comparable in the two treated pigs, the relative weight of mesenteric and colonic nodes in female 4243 was about half that in male 318.

TABLE 59

RELATIVE WEIGHT OF LYMPH NODES AND ORGANS (cg/kg BODY WEIGHT) OF INDIVIDUAL LYMPHOSARCOMA PIGS TREATED WITH CYTARABINE COMPARED WITH THREE NORMAL PIGS AND FOURTEEN UNTREATED CASES OF SIMILAR AGE :

	Normal Pigs Range of Three Controls	Cytarabine treated lymphosarcoma cases killed at end of treatment	Untreated cases Mean (range) of 14 cases
Age at Death (Days)	157 - 170	4243	318
		202	176
			182 (156 - 199)
Lymph Nodes:			
Mesenteric and Colonic	305 - 408	1426	2816
Gastric and Splenic	28 - 42	1128	1217
Bronchial Group	6 - 14	52	38
Total Splanchnic	361 - 449	2606	4071
			5040 (1595-12,229)
Head Group	24 - 32	106	113
Iliac Group	7 - 8	22	19
Prescapular	3 - 7	11	4
Precrural	2 - 5	15	9
Inguinal	11 - 14	30	29
Popliteal	1	2	3
Total Superficial	49 - 66	186	177
			266 (98.4 - 495)
Thymus	144 - 192	12	11
Spleen	122 - 432	1980	2013
Liver	2076 - 3306	3663	4579
Kidney	153 - 179	277	316
Adrenal	3 - 5	6	10
			25 (0 - 149)
			1500 (616 - 2702)
			6016 (3968 - 10,438)
			290 (211 - 400)
			10 (6 - 22)

Thymuses in both animals were involuted, spleens were large and livers fairly small, compared with mean weights from untreated cases.

Results of studies on deoxycytidine kinase (DCK) (Baxter, personal communication).

Initially, relative amounts of DCK in control and tumour lymph nodes were measured. The specific activity of DCK in nodes from tumour-bearing animals was up to four times greater than that in lymph nodes from the normal pigs.

However, kinetic studies performed on partially purified DCK indicated a K_m (dissociation constant) of $14.4 \mu M$ for the normal enzyme and $55.8 \mu M$ for the tumour enzyme. As the K_m is inversely related to the affinity of the enzyme for the substrate, it was concluded that the tumour enzyme would be less effective than the control enzyme in activating cytarabine. This obviously lessened any therapeutic advantage of having higher levels of the enzyme in the tumour.

DISCUSSION

The effects of subcutaneous twice daily cytarabine on these four lymphosarcoma cases were not dramatic. Two pigs improved in clinical condition, one neither improved nor deteriorated but had a mild attack of diarrhoea, and the fourth deteriorated and suffered from almost constant diarrhoea. Diarrhoea is recognised as a side-effect of cytarabine therapy in man (Upjohn Limited, Product Information Leaflet), but both these pigs had suffered from diarrhoea before cytarabine therapy, and therefore the drug may not have been responsible.

Shrinkage of superficial lymph nodes occurred in all four pigs, ranging from 20 to 40%, indicating an effect on the tumour. However, it is doubtful if this could be described as a partial remission, even temporarily. Abnormal cells never completely disappeared from blood smears, even in pig 318 in which the effect on WBC counts was most pronounced. The effects on the red cell pictures suggested that the pig which showed most improvement with therapy may have been the female 4243, and this was also suggested by the relative weight of the mesenteric and colonic lymph nodes at post-mortem. Although relative weights are not available for 4248 and 317, the post-treatment laparotomies showed that any effects on the mesenteric nodes of these two pigs were marginal.

Cytarabine has been used, in combination with other drugs (usually vincristine, cyclophosphamide and prednisolone), to treat lymphosarcoma in dogs (Theilen et al., 1977, MacEwen et al., 1977, Crow et al., 1977, and Owen and Bostock, 1978). It has never been reported as a single agent for the treatment of lymphosarcoma in dogs, but pharmacokinetic studies have been carried out in normal dogs (Ho, Carter, Loo, Abbot and McBride, 1975). These authors showed that tissue levels of cytidine deaminase, which converts cytarabine to uracil arabinoside, were very low and that the initial plasma half-life was 40 minutes, which is longer than in man. This information is not available in pigs, and Dr. Baxter and his co-workers have not yet investigated cytidine deaminase levels, therefore several theories may be put forward to explain the disappointing results of this experiment.

(1) Cytarabine is a cell-cycle specific agent, which acts best in rapidly-growing cells. It has been shown in many advanced tumours that the growth fraction is fairly small, many of the cells being in a resting phase. The growth fraction may be increased by reducing the tumour burden, using cell-cycle non-specific agents first, or in combination (Schabel, 1975). This may explain why cytarabine tends to be more effective in combination than as a single agent.

(2) Cytarabine is likely to have a short plasma half-life, and would probably have been more effective if it

could have been given more frequently, or by infusion, but this latter method was not practicable in our experiments.

(3) Tumours commonly become resistant to cytarabine. The mechanisms by which this occurs are not fully understood, but two possibilities have to be considered. Firstly, the tumour may increase manufacture of cytidine deaminase (though Smyth et al., 1976, did not find this to be the case in human acute myeloid leukaemia). Cytidine deaminase may be inhibited in vivo by an agent called tetrahydrouridine (THU). It would have been interesting to treat an animal with cytarabine plus THU. Secondly, the normal substrate with which cytarabine triphosphate competes is deoxycytidine triphosphate. Some tumours can respond to cytarabine triphosphate by expanding pools of deoxycytidine triphosphate, and so overcoming the DNA polymerase inhibition (Baxter, personal communication).

(4) The dose level may not have been adequate. This is not a likely possibility because the experiment using normal pigs showed that bone marrow depression occurred during the first 14 days of treatment before the dose was increased, and at least two of the lymphosarcoma pigs (317 and 318) showed depression of neutrophil and RBC counts, PCV and Hb during therapy. However, it has been suggested (Kremer, 1975) that in human acute myeloblastic leukaemia, severe bone marrow hypo-

plasia may be a requirement for a complete remission, and if this was also true in hereditary porcine lymphosarcoma, this could have been achieved by prolonging the treatment. According to Capizzi, Keiser and Sartorelli (1977), once an initial threshold of marrow toxicity has been reached with a phase-specific agent, further toxicity is usually not dose-dependent but is related to the duration of exposure. The reason for this is thought to be as follows. It has been demonstrated in experimental systems that a significant fraction of haematopoietic stem cells are normally out of cell cycle in the Go state. However as continued therapy destroys the descendents of these cells, there is an increasing demand on the stem cells to enter cycle and replace the increasingly depleted functional cells. Entry of more stem cells into the cycle makes them sensitive to phase-specific agents. The early descendents of the stem cells are highly proliferative and these too will suffer a significant reduction in numbers.

CHAPTER VFURTHER STUDIESLeucocyte Chemotaxis in Hereditary Porcine Lymphosarcoma

In 1962, Boyden described a technique for the measurement of the capability of neutrophils to move towards a chemical attractant. This is known as chemotaxis. Since then many authors have used the technique or adapted it in attempts to discover why neutrophils and mononuclear phagocytes migrate towards areas of inflammation and what factors are involved in inhibiting this important function. The literature was reviewed by Wilkinson (1974).

In recent years there has been considerable interest in defects of the immune system in cancer patients as many appear to be more prone to infections than normal. Chemotaxis is only one aspect of the response of the body's defense mechanisms and to be able to fully assess neutrophil and monocyte or macrophage function, one must also investigate phagocytic and bactericidal ability. However these functions are dependent for their success on the ability of the cells to migrate. Snyderman, Dickson, Meadows and Pike (1974) described deficient monocyte chemotaxis in patients with various types of cancer and suggested that this might be one reason why tumour cells are allowed to multiply rather than being killed by cellular immune responses. Later Snyderman and Pike (1976) reported isolation of a "chemotaxis inhibitor" from cancer patients. McVie (1978) described depressed monocyte chemotaxis in

lymphoma patients. In 16 out of 22 patients, there was a reduction of more than 20% compared with controls. Mean depression in the patient group was 35% but was greater in patients with advanced disease. There was no relationship between chemotactic ability and age, histological type of lymphoma, presence or absence of systemic symptoms and absolute blood monocyte counts. Results tended to become nearer to control results after therapy. Chemotherapy tended to depress monocyte chemotaxis but there was a wide scatter in the results.

There has been little investigation of the effects of individual anticancer drugs on chemotaxis. It is frequently stated that corticosteroids depress cellular defense mechanisms. Rinehart and LoBuglio (1973) investigated the effect of incorporating hydrocortisone with the medium in which the cells were suspended. They found that monocyte chemotaxis and bactericidal ability were markedly reduced, though there was no effect on neutrophil chemotaxis. However, when Rinehart, Sagone, Balcerzak, Ackerman and LoBuglio (1975) investigated chemotaxis of blood leucocytes from patients treated with oral prednisone, given every 12 hours for 3 days, the results were quite different. Monocyte chemotaxis was increased.

The principle of the method of Boyden (1962) was well explained by Wilkinson (1974). Chambers are

used similar to those designed by Boyden (See Plate 24).

There are two compartments in each chamber, the lower one containing the chemotactic substance and the upper one the cell suspension, separated by a piece of filter which has micropores of an appropriate size small enough to prevent the cells from simply falling through but large enough to enable them to squeeze through by active movement.

Distance migrated by the cells has been shown to be dependent on time allowed for migration, the absolute concentration of the chemotactic substance, the concentration gradient of the chemotactic substance across the filter and the strength of the particular attractant used. Casein is an example of a strong attractant but other substances such as bacterial filtrates, lymphoid culture supernatants or cobra venom may be used. There are optimum conditions of temperature and pH and an optimum concentration of chemotactic substance. The dose response curve reaches a maximum and then begins to decline perhaps because receptors on the cells become saturated or because the metabolic processes required for migration become exhausted. Chemotactic substances may be simply metabolic stimulators but this does not explain the directional migration. Gravity has no effect - the cells move uphill as readily as downhill - and if the gradient is reversed, the cells have been shown to change direction (Wilkinson, 1974).

It was decided that it would be interesting to investigate monocyte and neutrophil chemotaxis in lymphosarcomatous pigs compared with normal pigs, and the effects of prednisolone therapy in lymphosarcoma cases.

MATERIALS AND METHODSMaterials

Reagents required were as follows:-

(1) Phosphate buffered saline (PBS)

2.72g of KH_2PO_4 and 7.095g of $Na_2HPO_4 \cdot 2H_2O$ were weighed out. The KH_2PO_4 was dissolved in distilled water and made up to 100ml. The $Na_2HPO_4 \cdot 2H_2O$ was dissolved similarly and made up to 250ml.

These solutions were mixed in the ratio of 3:7, and 10ml of the mixture was added to 990ml of normal saline.

(2) Hepes buffer 1 molar pH 7.3 (Gibco, Bio-Cult, Glasgow).

(3) Medium 199, 10 times concentrated, (stock medium) with Hank's salts and L-Glutamine without $NaHCO_3$ (Gibco BioCult, Glasgow).

(4) 4N NaOH. This was made by dissolving 4g of NaOH and making up to 25ml with distilled water.

The working medium 199 was made up as follows:-

Medium 199 (conc.) - 10ml.

Distilled water - 86ml.

* Hepes/NaOH - 4ml.

* This was made by adding 5.5ml 4N NaOH to 100ml Hepes buffer.

The working solution pH was checked and brought to 7.0, if too alkaline, with concentrated hydrochloric acid.

These solutions were sterilised, by autoclavation at 15 lb for 10 minutes and stored at 4°C.

(5) Casein (BDH)

This was prepared as a concentration of 10mg/ml, 200mg was dissolved in 20ml of distilled water by raising the pH to 11.0 with N NaOH in a water bath at 56°C. Once the casein was dissolved, the pH was gradually reduced to 7.0 using phosphoric acid. The solution was frozen at -40°C in 1ml aliquots, and was replaced after one month's storage.

The working solutions were 1mg/ml and 0.5mg/ml casein, and occasionally 2mg/ml, in Medium 199. Two aliquots were added to 8ml Medium 199 and Hepes, prepared as described above, to make 2mg/ml casein. An aliquot of this was diluted with equal parts of the medium to make 1mg/ml casein, and repeated to make 0.5mg/ml.

(6) Ficoll (Ficoll 40a- Pharmacia Fine Chemicals, Uppsala, Sweden).

(7) Hypaque (Hypaque Sodium, Winthrop Laboratories, Surrey).

Preparation of Ficoll/Hypaque was carried out as follows:-

To make a 9% solution of Ficoll - a convenient volume was 250ml.-

22.5g of Ficoll was weighed out and dissolved in distilled water. Placing in a water bath at 56°C aided solution. Distilled water was added to make up to 250ml. To make a 34% solution, 34g of Hypaque was weighed out, dissolved in distilled water and made up to 100ml. The two solutions were mixed in the proportions of 24 parts Ficoll to 10 parts of Hypaque. The specific gravity of the

resulting mixture should be 1.076 - 1.078, and if it was too high, distilled water was added to bring the specific gravity to the correct reading. This was dispensed in universal containers in volumes just greater than 20ml, and sterilised by autoclavation at 15 lb pressure for 10 minutes.

(8) Gelatin

This was prepared by dissolving 2.5g gelatin powder in PBS in a 56°C water bath. The solution was made up to 100ml with PBS and dispensed in sterile plastic universal containers in volumes just greater than 20ml. These were stored in the refrigerator and liquified when required by placing them in a 37°C incubator half an hour before use.

Apparatus Used

(1) Chemotaxis filter chambers. The number required was 12 but 16 were used on some occasions for monocytes. Half of the chambers were used for a lympho/^{sarcoma} pig's cells and half for those of a control pig. See Plate 24.

The cells were tested as follows:-

- (a) Two chambers with no chemotactic attractant.
- (b) Two chambers with 0.5 mg/ml casein.
- (c) Two chambers with 1mg/ml casein.
- (d) For monocytes, two chambers with 2mg/ml casein.

(2) Filters, pore size 8×10^{-6} m (μ m) for monocytes,

3 μ m for neutrophils (Millipore, Molsheim, France). Pieces of filter of suitable size to fit the chambers were cut out and placed in position using Millipore forceps. The membranes were then kept in position by screwing in the screw insert using a metal key. The filter area exposed to the test cells was therefore similar to the area of the lumen of the screw insert.

Methods

Monocytes

For each run, a sample of 20ml of venous blood was collected from one normal pig and one lymphosarcoma pig of the same sex and approximately the same age. The blood was taken from the anterior vena cava as described in the General Methods section, and 2ml was put into EDTA for WBC and differential counts. The remainder was defibrinated, by gentle shaking, in glass universal containers with 10 to 15 glass beads in each. Probably some monocytes were lost using this method because of their tendency to adhere to glass. However heparin and acid citrate dextrose had been tried previously as anticoagulants and found to be unsuitable. This was because large numbers of thrombocytes were mixed with the separated mononuclear cells, obscuring the filters and occasionally causing the cells to aggregate forming large cell and thrombocyte masses which prevented migration of the monocytes. Defibrination effectively removed most of the thrombocytes.

In the laboratory, the blood was removed from the universals by Pasteur pipette, leaving behind the beads with the fibrin clot adhering to them. Unless the lymphosarcoma pig was very anaemic, the blood was diluted with an approximately equal volume of PBS. The diluted blood was carefully layered on to Ficoll-Hypaque in plastic conical centrifuge tubes, about 5ml of each. The tubes

were spun at 2000G for 15 minutes in a bench centrifuge. The layer of mononuclear cells just above the interface was removed from each tube along with some plasma using a Pasteur pipette and the cell suspension put into Taylor tubes. They were spun down at 400G for 10 minutes, the supernatant was removed, the cell button was gently resuspended and PBS was added. This procedure was repeated for two PBS washes and finally the cells were resuspended in the Medium 199/Hepes working solution.

The number of cells/ml present was calculated after counting the number in a white cell dilution using the Coulter counter and diluter (as previously described in the General Methods section for whole blood). The cell suspensions were diluted until there were approximately 2.5×10^6 cells/ml.

Neutrophils

Blood samples of 20ml were taken as for monocytes, but into "Vacutainers" containing acid citrate dextrose. The samples were mixed well.

In the laboratory, the samples were centrifuged at 500G for 20 minutes in a bench centrifuge. The thrombocyte-rich plasma and buffy coat layer of mainly mononuclear cells were removed using a fine pasteur pipette. The remainder was mixed with liquified 2.5% gelatin in PBS and the erythrocytes were allowed to sediment at an angle in an incubator at 37°C for 45 minutes to one hour. The supernatant was removed and centrifuged at 400G for 10

minutes at room temperature. The neutrophils were then washed, suspended in Medium 199 and counted as described above. It was found that the optimum cell concentration was 2×10^6 cells/ml.

The cell types present in the cell suspensions obtained by these methods were investigated using a "Cytospin" centrifuge. Using this apparatus, one or two drops of fairly concentrated cell suspension could be spun on to microscope slides. These preparations were stained with Leishmann's stain, as previously described for blood smears but mixing the stain well every few minutes, so that the cells would take up stain evenly.

The mononuclear cell suspensions contained lymphocytes, tumour cells in those from the lymphosarcoma cases, and monocytes, with relatively few neutrophils and eosinophils. The neutrophil suspensions contained eosinophils and a few mononuclear cells. Contamination with red cells was variable depending on the success of the gelatin sedimentation but, unless very numerous, they did not affect the reading of the filters. Absolutely pure cell suspensions were not necessary because the different types were quite easy to identify under the microscope.

The cells suspensions were used to fill the chemotaxis chambers as follows:-

Medium or medium containing casein was introduced into the horizontal part of the chamber and up to the level of the underside of the filter. Then, using two

Pasteur pipettes, the test cells were added to the lumen of the screw insert above the filter, and medium (or medium plus casein) to the other side of the chambers. Simultaneous filling of both sides was important so that the pressure was equal on the top and underside of the filter. Both sides were taken as filled when menisci developed above the level of the top of the chamber. This allowed for evaporation of moisture during the incubation period.

After 2 hours incubation at 37°C for monocytes, 1 hour for neutrophils, in a sandwich box containing moist tissues, both sides of the chambers were emptied simultaneously with Pasteur pipettes. Each chamber was then tilted so that an air bubble displaced the fluid in the horizontal part and this was sucked off. The insert was unscrewed and finally the filter was removed gently using Millipore forceps and placed in 98% ethanol. The filters were either left in this overnight, or for 10 minutes before proceeding with the staining of the filters. Ice cube trays were used for the staining process which was as follows:-

90% ethanol	-	2 to 5 minutes
75% ethanol	-	2 to 5 minutes
50% ethanol	-	2 to 5 minutes
Distilled water	-	2 to 5 minutes
Mayer's Haematoxylin	-	4.5 minutes
Tap water	-	10 to 30 minutes
50% ethanol	-	2 to 5 minutes

75% ethanol - 2 to 5 minutes
90% ethanol - 2 to 5 minutes
98% ethanol - 2 to 5 minutes
Xylol - overnight

98% ethanol was used instead of absolute ethanol as the latter tended to dissolve the filters.

The filters became almost transparent in the xylol and were mounted next morning on microscope slides in D.P.X.

Examination of the Membranes

This had to be carried out within 48 hours as the membranes tended to become opaque after this time.

Readings were carried out on a Leitz microscope using the x 40 dry objective.

The filters were scanned initially using a low power objective to locate the main cell population.

Using the fine adjustment, the top of the filter was located by focussing on cells which had not migrated into the filter. The number of the Vernier scale on the fine adjustment was noted. The focus was adjusted down through the filter until the plane containing the last two or three cells was found. The fine adjustment Vernier travel between the first and second readings was noted. The reading was carried out five times on each filter and then another five times by a second observer. Means and standard deviations on the readings were calculated for no casein, 0.5mg/ml, 1mg/ml and 2 mg/ml casein for each pig and the results plotted on linear graph paper.

The method was similar to that used by McVie (1978) with the following modifications.

- (a) The Ficoll/Hypaque mixture used for monocytes was one which had been found to be suitable for pig cells. McVie (1978) used a Ficoll/Triosil mixture.
- (b) Almost all the anticoagulants tried, including heparin, allowed thrombocyte aggregation. Acid citrate dextrose caused fewest problems and was suitable for the neutrophil separation but, despite the risk of losing many of the monocytes by adherence, it was necessary to defibrinate blood for mononuclear separation. Gelatin was used for sedimentation of RBCs instead of dextran (Henson, Johnson and Spiegelberg, 1972).
- (c) Absolute alcohol tended to dissolve the filters and so 98% was used.
- (d) The cell concentration for monocytes was increased to offset the smaller numbers of monocytes present.

McVie (1978) counted monocytes in the cell suspensions in a haemocytometer but it proved very difficult to identify pig monocytes, particularly in suspensions from the lymphosarcoma cases in which there were many large mononuclear tumour cells. These were not a problem in the test because neither the lymphocytes nor the tumour cells migrated, therefore attempts to isolate monocytes were abandoned and the cells were counted using the Coulter counter to speed up this rather slow technique.

(e) In the monocyte tests, the maximum casein concentration was increased to 2mg/ml.

RESULTS

The results of the 20 test runs are shown in Figures M1 to N10.

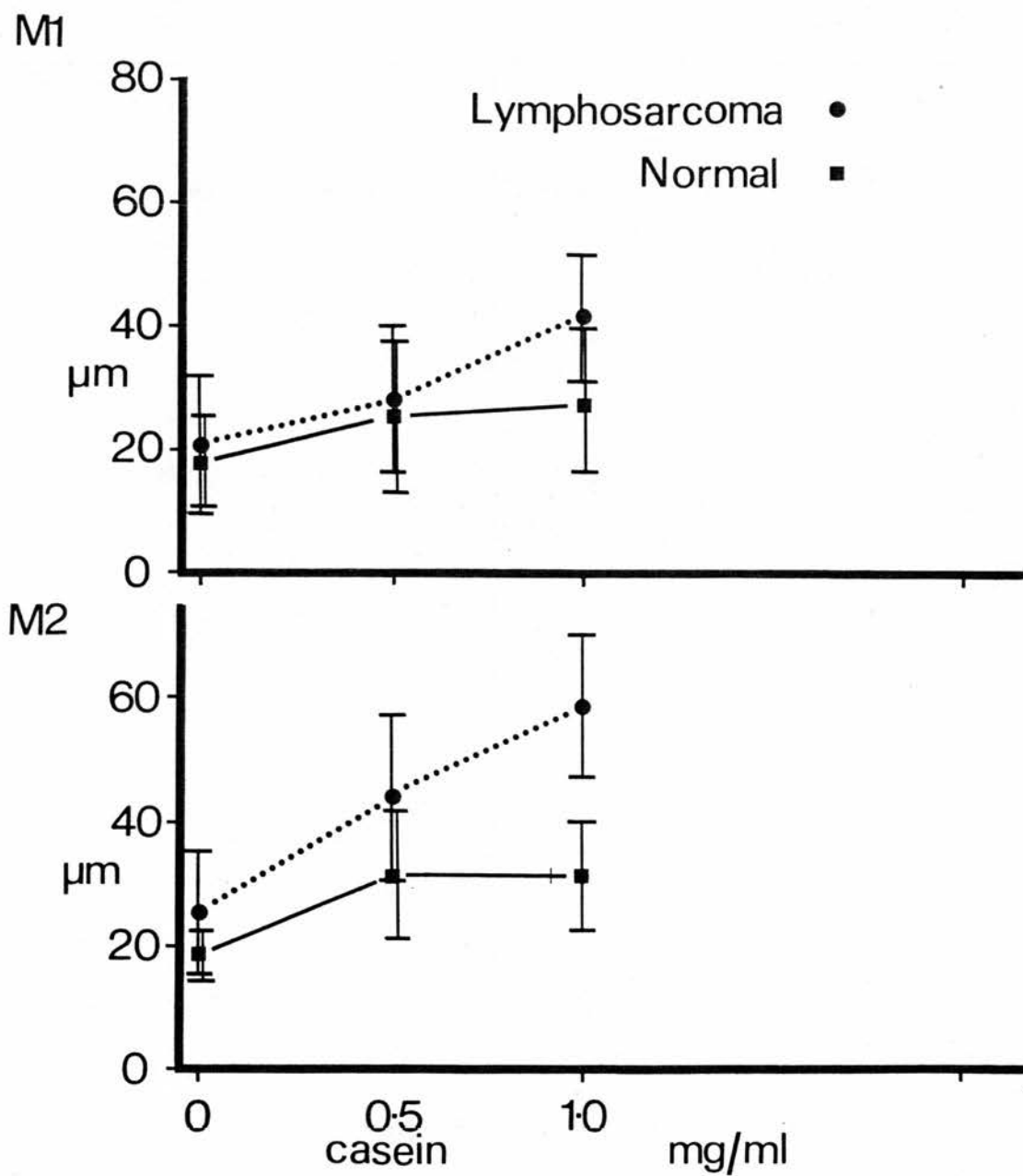
Untreated pigs - It can be seen from all the Figures that monocytes and neutrophils migrated a short distance into the filters even in the absence of casein. In tests M1, M2 and M3 the normal pigs' monocytes did not migrate very much further at 0.5 and 1.0mg/ml casein and so another casein concentration, 2mg/ml, was introduced. The distance migrated by the normal pigs' monocytes increased greatly at this concentration. In Test M3 both filters with the lymphosarcomatous pig's cells were spoiled at 2mg/ml. In all monocyte Test runs (M1 to 5) it can be seen that at 0.5 and 1mg/ml the lymphosarcomatous pig's cells migrated further than those of the normal pig, particularly in Tests M2 and M5. At 2mg/ml, this was reversed (See Tests M4 and M5).

Neutrophil chemotaxis in all five tests (N1 to N5) showed a fairly good dose-related response and 2mg/ml casein was considered to be unnecessary. In Tests N1 and 2, the lymphosarcoma case's cells showed a slightly poorer migration than those of the normal but the standard deviations of the readings overlap. In test N3 the distance migrated by the lymphosarcoma pig's neutrophils was slightly greater than the normal and in Test N5 this was also the case at 0.5mg/ml casein. In Test N4, the chemotaxis of the lymphosarcoma neutrophils appeared to be

depressed at both 0.5 and 1mg/ml.

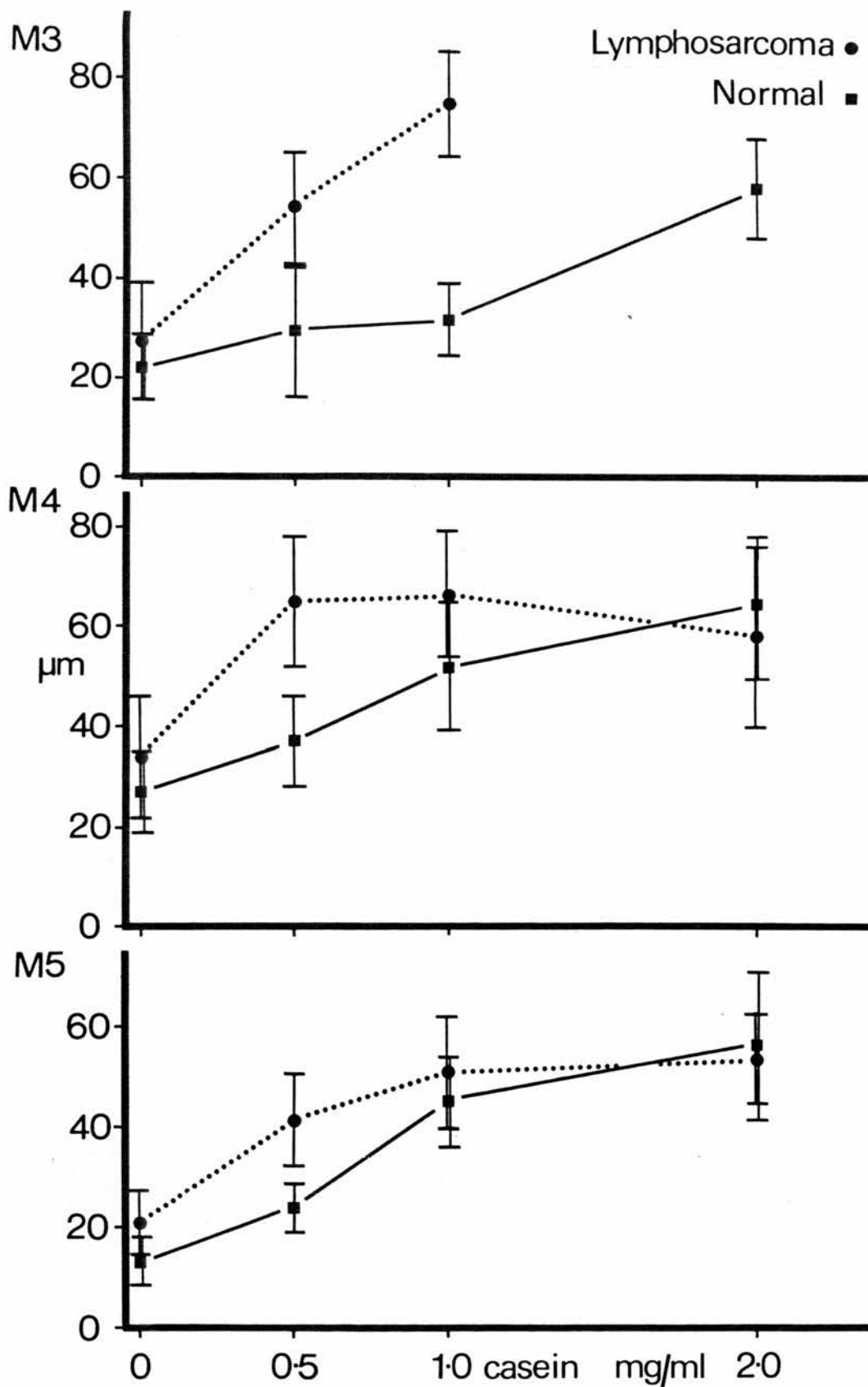
Prednisolone - treated pigs - Monocyte chemotaxis of the lymphosarcoma case in Test M7 was poorer than that of the normal at 1mg/ml casein and in Tests M9 and 10 there was virtually no difference. However in every test (M6 to 10) there was still a tendency for the migration of the lymphosarcoma pig's monocytes to exceed that of the normal pig, though the standard deviations of the readings overlap.

In the neutrophil tests (N6 to N10) the results were very variable. In tests N6 and N7, and at 0.5mg/ml casein in test N8, chemotaxis in the lymphosarcoma cases was apparently depressed. In Test N9 there was no difference and in Test N10 at 0.5mg/ml the distance migrated by the normal pig's cells appeared to be abnormally low. This may well have been due to technical difficulties.



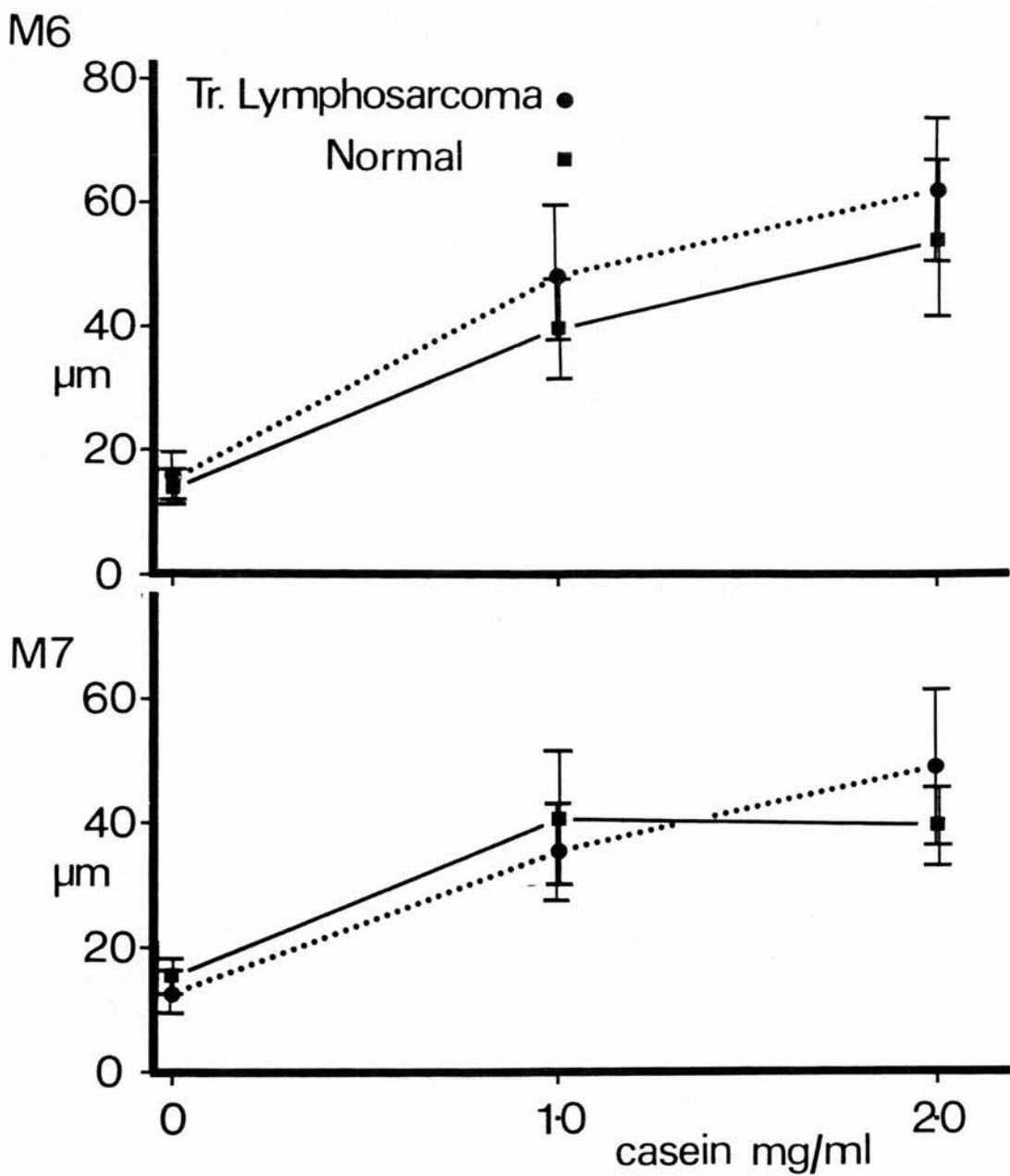
FIGURES M1 and M2

Distance migrated through filters by monocytes from two lymphosarcoma cases and two normal pigs.



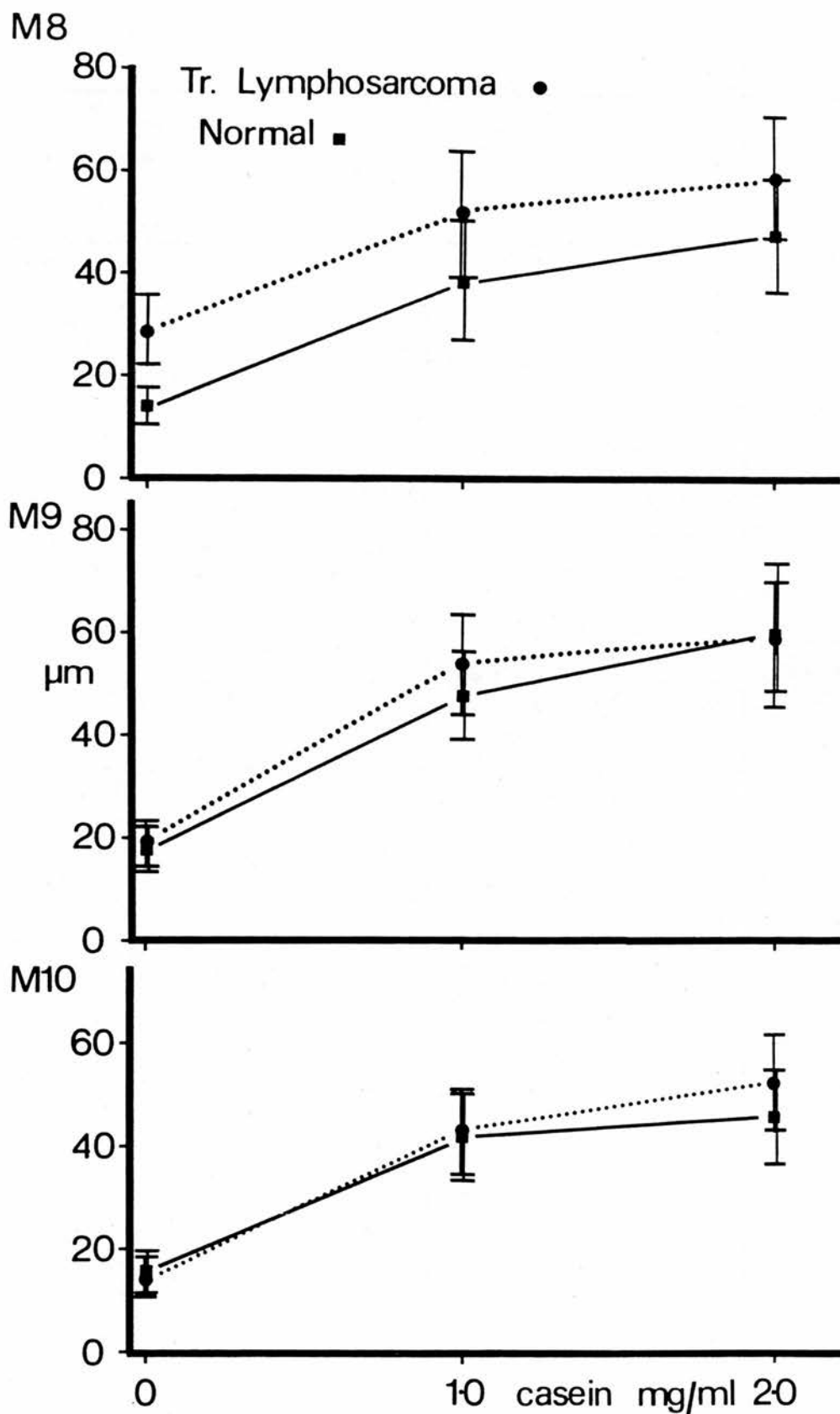
FIGURES M3, M4 and M5

Distance migrated through filters by monocytes from three lymphosarcoma cases and three normal pigs.



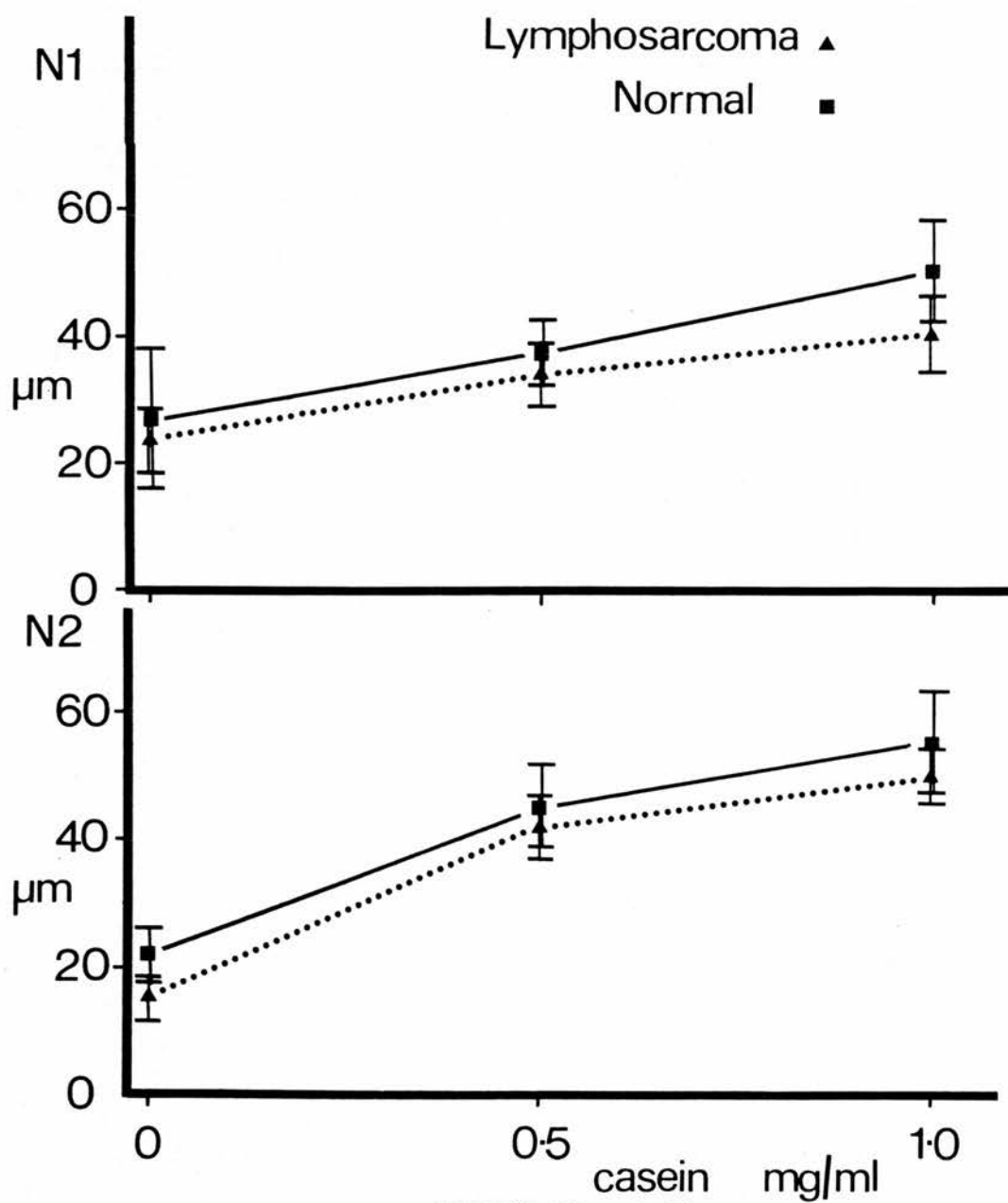
FIGURES M6 and M7

Distance migrated through filters by monocytes from two prednisolone - treated lymphosarcoma cases and two normal controls.



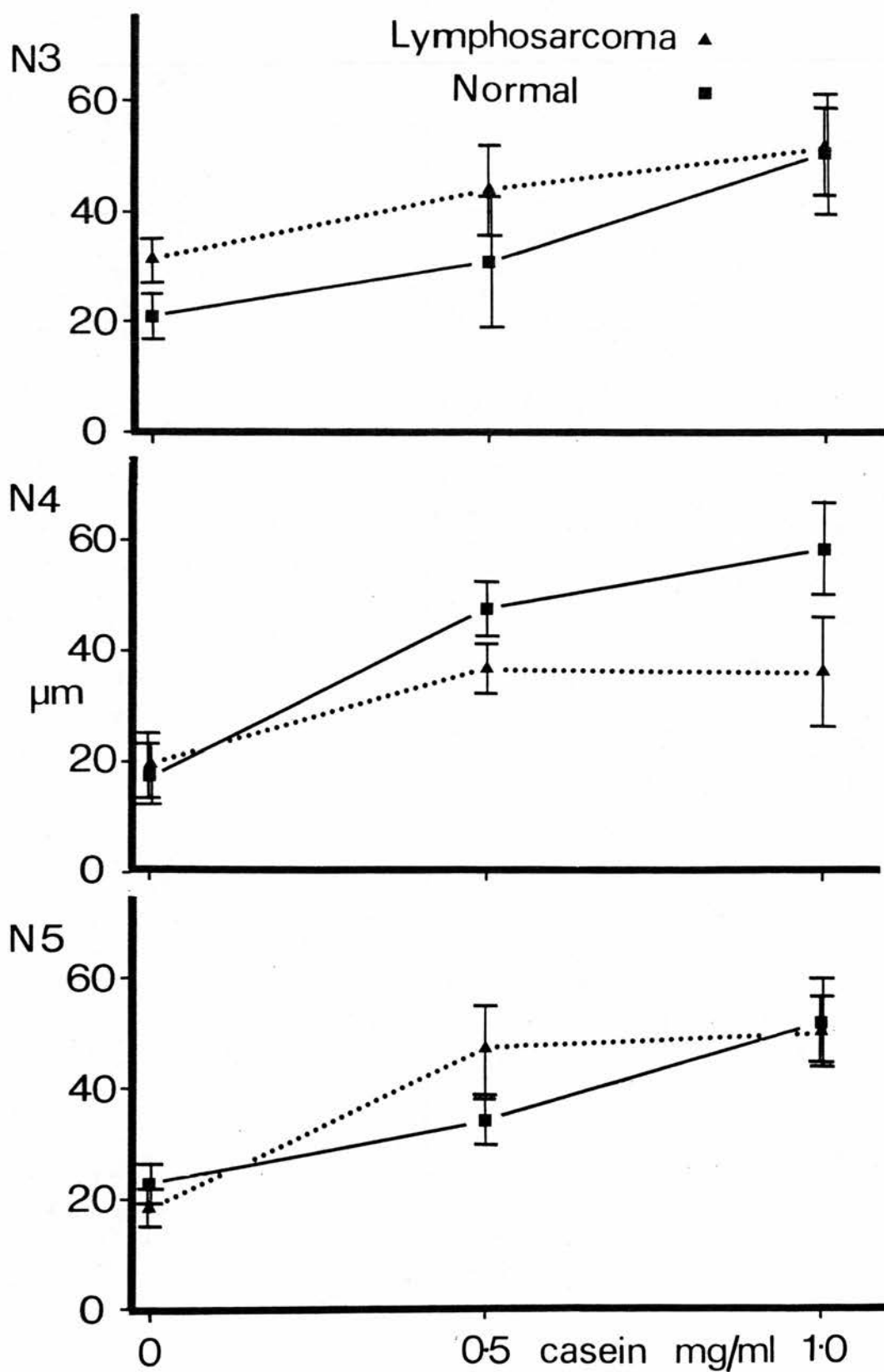
Figures M8, M9 and M10

Distance migrated through filters by monocytes from three prednisolone-treated lymphosarcoma cases and three normal controls.



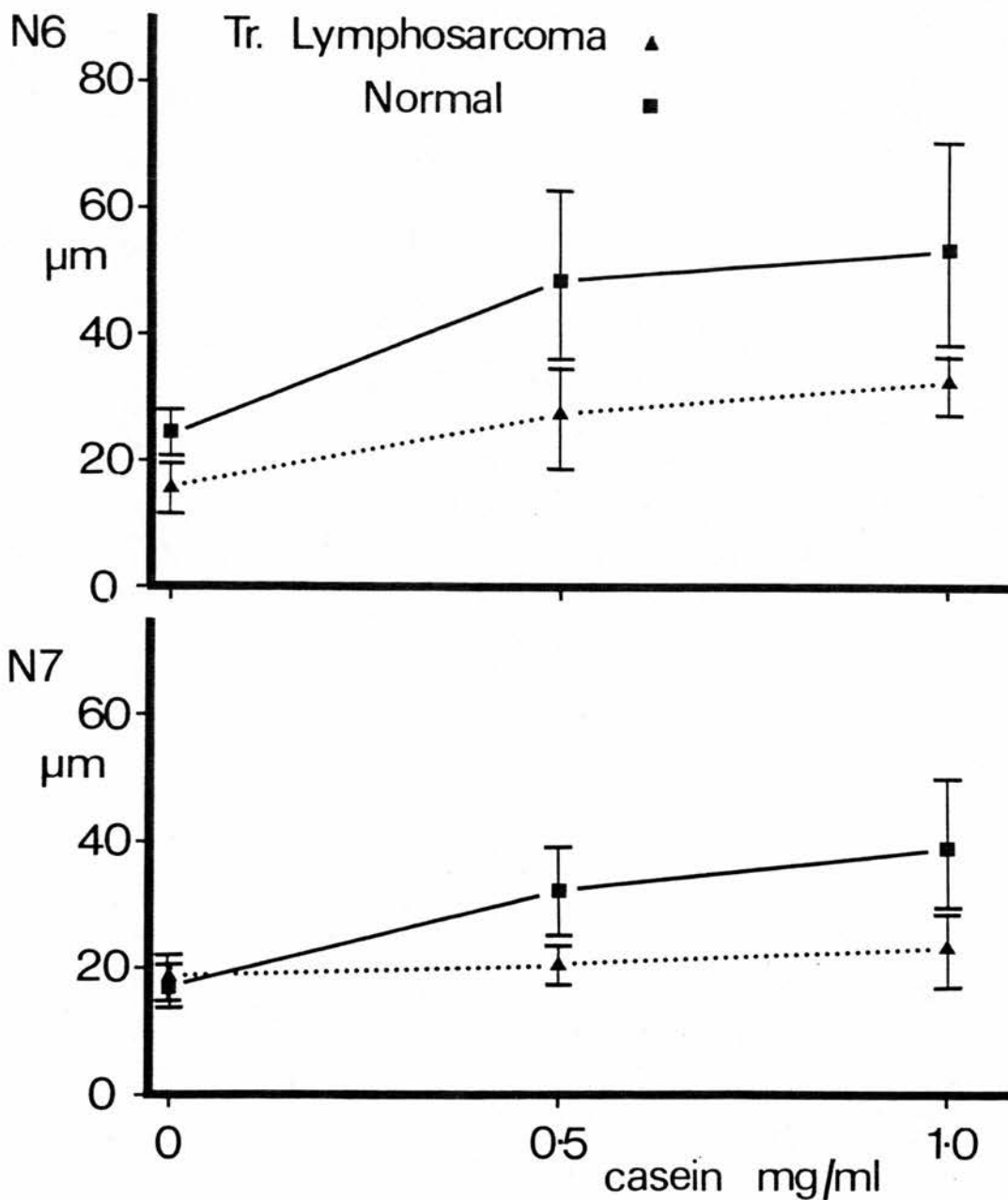
FIGURES N1 and N2

Distance migrated through filters by neutrophils from two lymphosarcoma cases and two normal pigs.



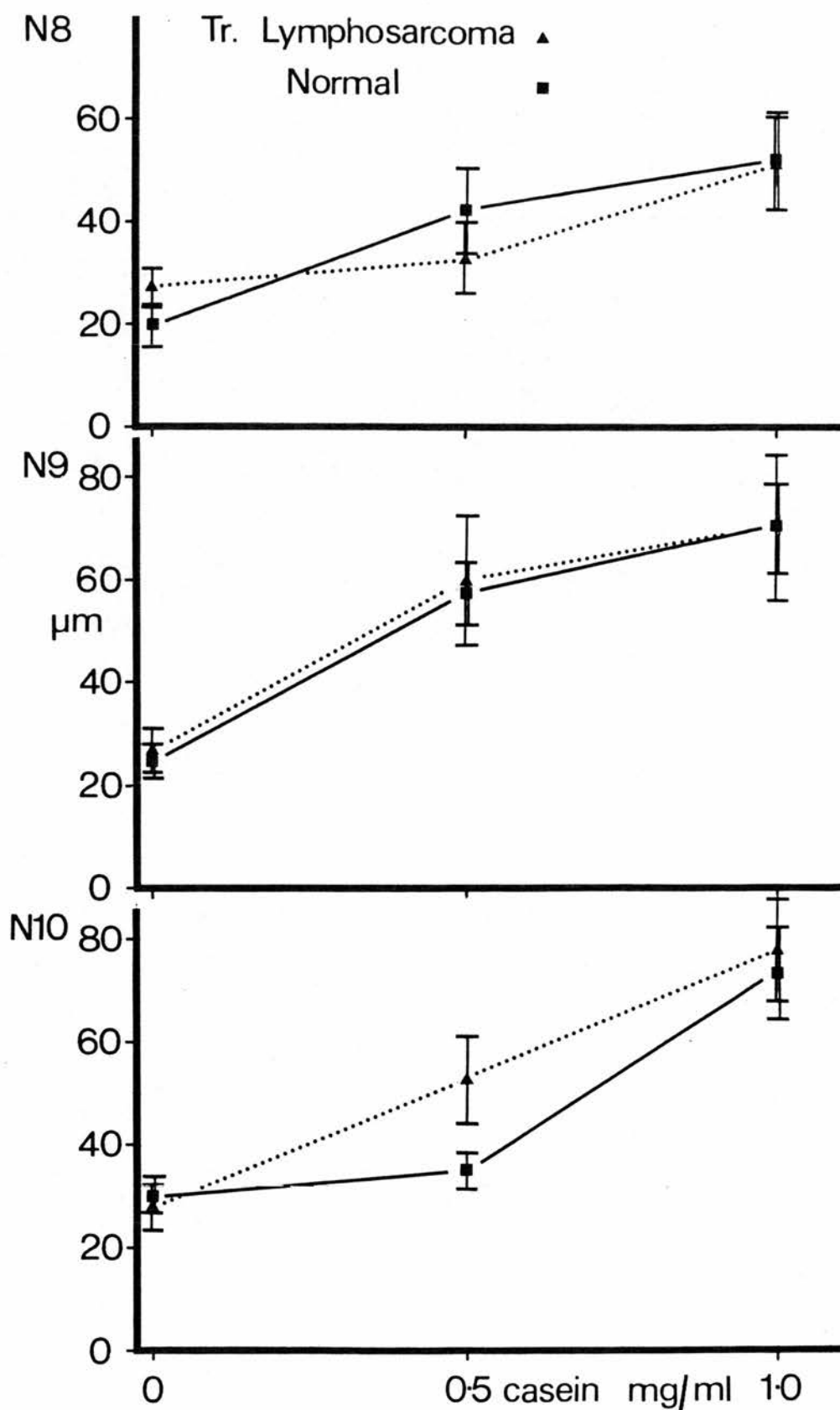
Figures N3, N4 and N5

Distance migrated through filters by neutrophils from three lymphosarcoma cases and three normal pigs.



FIGURES N6 and N7

Distance migrated through filters by neutrophils from two prednisolone-treated lymphosarcoma cases and two normal pigs.



FIGURES N8, N9 and N10

Distance migrated through filters by neutrophils from three prednisolone-treated lymphosarcoma cases and three normal pigs.

DISCUSSION

The results of 10 tests of monocyte chemotaxis suggest that it is not depressed in lymphosarcomatous pigs and at lower concentrations of casein these monocytes may be much more active than normal monocytes, especially in untreated cases. In the prednisolone treated cases monocyte chemotaxis was much closer to that of the normal pigs.

The observation that the monocytes of lymphosarcomatous pigs may be more active than those of normal pigs may be supported by a single test of phagocytic ability which was investigated by the hexose monophosphate shunt method by Dr. Anne Naysmith, Department of Therapeutics, Edinburgh Royal Infirmary. The activity of this enzyme in monocytes is related to phagocytic ability and is measured by determining the amount of $^{14}\text{CO}_2$ generated from 1- ^{14}C glucose in the resting state and when phagocytosing latex particles. The lymphosarcoma monocytes were shown to be more active than normal both in the resting state (350 counts/minute compared with 82 counts/minute) and at 1 in 100 dilution of latex particles (408 counts/minute compared with 160 counts/minute).

These results do not agree with the observations of Snyderman et al., (1974) and McVie (1978) in human patients. The tendency for chemotaxis to return to normal when patients were in remission was noted by McVie (1978). It would have been interesting to compare the effects of

prednisolone with other agents both in normal and lympho-sarcomatous pigs.

The results of the 10 tests of neutrophil chemotaxis are very variable but it appears that in certain lympho-sarcomatous animals this function may be depressed and this was also true in two out of the five prednisolone treated animals (N6 and N7). These were the same cases used for the monocyte chemotaxis (M6 and M7) and it is interesting to note that although neutrophil chemotaxis was depressed, monocyte chemotaxis was not. McVie (1978) suggested that there may be some mechanism whereby monocytes compensate for defects in neutrophil numbers or functions.

All of the results are very dependent on the technical accuracy of the technique. It was complicated and very time-consuming, with many stages where errors could occur and a considerable amount of practice was required before the manual dexterity necessary for filling the chambers was acquired. Nevertheless, the Boyden chamber technique is undoubtedly much easier to perform than other methods, such as that using sawn-off syringe barrels with filters stuck to them (Wilkinson, 1974).

CONCLUSIONS

The effects of one of each of the four major classes of anticancer chemotherapeutic agents have been investigated in normal pigs and pigs with hereditary lymphosarcoma.

The first agent, a glucocorticosteroid, was prednisolone. Administered daily intramuscularly in normal pigs, it produced mild clinical effects at a dose level of 6mg/kg, which included polydipsia, polyuria, abdominal muscle weakness, depression of weight gain and suppression of skin delayed hypersensitivity reactions. Other effects noted were a fall in lymphocyte counts and a reduction in relative weights of thymus and lymph nodes at autopsy. These effects are similar to those which occur during corticosteroid therapy in man and place the pig in the group of corticosteroid resistant species which includes man, the ferret and the guinea-pig. In cases of hereditary lymphosarcoma, prednisolone therapy, whether administered intramuscularly or orally, rapidly produced a good partial remission, though there were signs that this may only have been temporary. Appetite and fitness improved, though growth rate did not, there was rapid shrinkage of superficial lymph nodes and skin delayed hypersensitivity tests showed increased responses. Absolute mononuclear ^{few} cell counts were dramatically reduced and/abnormal cells were seen in blood smears by the end of treatment.

The anaemia and thrombocytopenia, typical of the advanced lymphosarcoma case, improved greatly and these improvements were maintained for a short time after therapy was stopped. Marked reductions in relative weights of lymph nodes, spleen and adrenal glands were shown at autopsy. These findings correspond well with the reported effects of prednisolone therapy of lymphoid tumours in human patients. However there were certain differences between the results described here and reports of some effects of corticosteroids in man and other animals, including pigs. Neutrophilia was observed in two animals only, the lymphosarcoma cases which were dosed orally, and these had suffered from severe diarrhoea. Eosinopenia, a classic feature in man, was not detected, though no special counting technique was employed. Anticipated changes in serum levels of sodium, potassium, chloride, calcium and inorganic phosphate did not occur. The only animal to show gastric lesions at autopsy was one of the orally treated cases. Nevertheless, several interesting features came to light. These were the marked increases in serum albumin and decreases in serum globulins, the fluctuations in magnesium levels, the correction of energy to DNFB in the lymphosarcoma cases which contrasted with the suppression of response in the normal pigs, and the histological evidence of different degrees of tumour suppression in different organs and even within the same organ and infiltration of the ovaries which never occurs in untreated cases.

The second agent used was the antitumour antibiotic adriamycin which was administered intravenously every 3 weeks. It appears from the results described in this thesis that there are many similarities between the effects of adriamycin in man and its effects in the pigs, which were cumulative. Bone marrow depression, gastrointestinal damage, alopecia, stomatitis with ulceration, sclerosis of blood vessels and tissue damage and the possibility of cardiotoxicity are features in both species. A dose-level of 4mg/kg caused death within 8 weeks and 2mg/kg within 16 weeks in normal pigs, but few clinical effects were noted in pigs given 1mg/kg. In cases of hereditary porcine lymphosarcoma, a dose level of 2mg/kg was associated with severe liver dysfunction which has not been reported in man as an effect of adriamycin alone, and death occurred rapidly in both the cases treated, though tumour suppression did occur. In the lower dose animals, a shrinkage of tumourous lymph nodes, a temporary reduction in numbers of abnormal cells in the blood and an improvement in body condition and weight gain provided objective evidence of a response to adriamycin therapy, though the response was not good enough to be described as a partial remission. In man remissions induced by adriamycin alone have been reported to be short but these animals showed no signs of development of tumour resistance after 5 months of therapy.

The third agent used was the alkylating agent cyclophosphamide which was also administered intravenously

every 3 weeks. In the experiment using normal pigs the only clinical effect noted was temporary pallor of skin and mucous membranes which corresponded with slight anaemia, in one animal given the highest dose of 80mg/kg. WBC counts, and neutrophil counts in particular, were severely depressed during the first 5 days after dosing but rapid recovery took place. This also occurs in man but several days later. Alopecia is common with this agent in man but was not seen in the pigs, though it did occur with the closely related agent ifosfamide. Haemorrhagic cystitis, also common in man, was not seen, though one low-dose animal showed mild bladder changes at autopsy. The experiment was disappointing in that it did not demonstrate many similarities between the responses of humans and pigs to cyclophosphamide. From the literature it appears that dogs resemble man more closely in this respect. One interesting feature was the depression of skin delayed hypersensitivity response and reduction in size of the thymuses of the treated pigs - apparently an effect on T cells by a drug which is reputed to act principally on B cells. Cyclophosphamide therapy of lymphosarcoma cases, at a dose level of 20mg/kg, which may have been too low, was not associated with any clinical signs of toxicity. WBC counts were depressed as in the normal pigs and abnormal cells disappeared from blood smears but this was only temporary. As with prednisolone, there appeared to be an improvement in skin delayed hypersensitivity responses. The females in the experiment responded moderately well, though not well enough for the

response to be called a partial remission, but the only treated male hardly responded at all as shown by relative lymph node weights at autopsy. It has been shown (unpublished observations) that untreated entire male cases do not live as long as female cases and, though the mechanism is not known, it is possible that sex also affects response to treatment in some way. Cyclophosphamide was much more useful as a component of a combination chemotherapy regime, but the results are not included here.

The fourth major group of anticancer agents are the antimetabolites and cytarabine was chosen from this group because initial studies had shown that in tumour tissue from the lymphosarcoma cases the specific activity of DCK, the enzyme which activates cytarabine, was much greater than in lymphoid tissue from the normal pigs. This activity was shown to be up to four times greater in lymphosarcoma tissue. In normal pigs a dose level of about 2mg/kg/day given subcutaneously twice daily resulted in progressive bone marrow depression and although the dose level was increased later the results were not dose-related. Recovery was rapid when dosing was stopped. All types of WBC, RBC and thrombocyte counts were affected. This also occurs in man, though lymphocyte counts are said to be barely affected. The only clinical effect noted was slight weight loss and the vomiting and diarrhoea, oral inflammation and ulceration, hepatic dysfunction, fevers and skin rashes reported as side-effects in man were not seen in the normal pigs. Two out of the four lymphosarcoma cases

treated with cytarabine suffered from diarrhoea but this may not have been caused by the drug. The results of therapy were disappointing. One male actually deteriorated and only one pig, the only female, showed any real evidence of improvement in body condition, haematological parameters and at autopsy. Though superficial lymph nodes decreased in size by between 20 and 40% the response, even in the female, was not good enough to be called a partial remission. Enzyme kinetic studies suggested that the advantages of high levels of DCK in the tumour tissue would be lessened because the tumour enzyme was less effective than the control enzyme in activating cytarabine. Nevertheless, further studies could be carried out to determine whether the response could be improved by alteration of dose-level and scheduling or whether the poor response was due to deactivation by cytidine deaminase which could be inhibited by administration of tetrahydrouridine. In man, cytarabine is most frequently used in cases of acute myeloid leukaemia, although it is often effective in lymphoid tumours which do not respond to standard therapy.

From the results of these trials, it appears that the pig may be relatively resistant to some of the toxic side-effects of anticancer agents. However, it must be remembered that many of the side-effects reported in humans only occur in a small proportion of patients treated with a given drug and it would be unwise to draw conclusions

based on experiments using such a small number of animals. The normal pig trials were carried out to establish a suitable dose-level for the lymphosarcoma cases and this objective was achieved. The most important conclusions concern the responses of the lymphosarcoma cases to therapy. It has been shown clearly that hereditary porcine lymphosarcoma is a valuable model for the chemotherapy of lymphoma in man and companion animals because of the vast amount of information which has been obtained from serial detailed studies on a few animals. This information is not only about different degrees of tumour suppression with different agents and schedules but also about aspects which are so important in clinical human or veterinary medicine but cannot be investigated easily using any other type of model i.e. the effects of the drugs on animals which are in a poor nutritional state, which show immunosuppression and which are prone to secondary infections.

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